

ON
CERTAIN BACTERIA
FROM THE
AIR OF NEW YORK CITY.

BY
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Submitted in partial fulfillment of the requirements
for the
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XII.—*On Certain Bacteria from the Air of New York City.**

BY HARRISON G. DYAR, A. M.

Read February 18, 1895.

It was suggested to me by Dr. T. Mitchell Prudden that a promising field for research existed in determining the identity of the bacteria commonly occurring in the air of New York.

Very early in the investigation a practical difficulty in the way of determining species became apparent. This resulted from the lack of any monographic treatment of the subject from the specific point of view. I find the same difficulty is met with by other investigators. Dr. Paul Schneider begins his inaugural address with these words: "Im Laufe der letzten Jahre sind nach und nach eine solche Menge verschiedener Bakterienarten beschrieben worden, dass es demjenigen, der sich nicht fortwährend damit beschäftigt, zur Zeit ganz unmöglich ist, sich in dieser Pflanzengruppe, die zwar arm an Formen, aber reich in Arten ist, zurecht zu finden."

It was thought that this difficulty could be overcome by the facilities possessed by the bacterial laboratory in the College of Physicians and Surgeons of Columbia College, with its considerable collection of living species, which might be directly compared with those obtained from the air. Further, a collection of fifty species was ordered from Král's bacterial laboratory at Prague with this special object in view. It was found, however, that the determinations of the species were not always authentic, as seen by the fact that when planted on the standard media many of them contradicted their published characters. The only cultures on which dependence could be placed were a few authentic ones which the college has received, identified by Dr. Sternberg, and those species from the college collection which Dr. Cheesman has had occasion to work out. Therefore, I have had to rely mostly on the published descriptions for identification of my cultures.

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For this reason, I have felt some hesitation about describing new species, but I see no other so satisfactory way of treating the undetermined cultures. The method of simply describing them without the application of a scientific name is eminently confusing, as I have experienced in going over the literature. I do not think it should be commended even though it may avoid the possible creation of synonymic names.

SPECIES AMONG THE BACTERIA.

The appearance of the new work on water bacteria* by the Franklands, with its appendix repeating the old descriptions of bacteria, brings up again the practical difficulty in the determination of species. There is nothing to be said of these descriptions which would not apply equally to those of any of the recent diagnostic treatises such as those of Eisenberg or Sternberg; all are in an equally unsatisfactory condition.

This condition is due not to a lack of discrimination on the part of the authors of these works, but to a faulty method of describing species which has come to be prevalent. The result is illustrated in Frankland's synoptic table, which, for example, contains ninety species under a single heading (bacilli which liquefy gelatin, p 396), these species only to be distinguished by laboriously reading through the several descriptions, many of which present no tangible points of difference. Dr. Sternberg, too, has made a valiant and praiseworthy effort to prepare a "bacterial diagnosis" (See Manual of Bacteriology, pp. 753-768), but the positive differential characters given in the several descriptions became exhausted long before he reached the separation of individual species.

Let us look into the matter a little further. La Semaine Medicale of June 16, 1894, contains an article by Drs. F. Helme and Paul Raugé, in which the authors review the characters which are in use to separate the genera and species of the bacteria. As they point out the ultimate characters used to separate species are physiological. The generic characters are morphological, but of such a nature as would scarcely be considered reliable among the higher forms. This results from the simple organization of the bacteria. As we proceed from the higher to the lower forms

* "Micro-organisms in Water," by Percy Frankland, Ph. D., and Mrs. Percy Frankland (1894).

of life, the organization becomes simpler, till with the bacteria we reach organisms which present extremely slight differences, so slight that morphology almost fails to furnish generic characters, and entirely fails to differentiate every species.

If conjugation is absent among the bacteria, and has never been present, the cells reproducing always by fission into equivalent parts, a condition of affairs should exist such as assumed by Weismann for the primitive form of life, and we should see all characters acquired by any species under the influence of external conditions, strictly inherited. In this case, there would be no such thing as species among the bacteria, merely an indefinite number of intergrading varieties. A given culture would probably show constant characters, but it would scarcely be expected that another culture would ever be found to show exactly the same characters.

But owing to the nature of the bacteria, dependent, for the most part, on previously prepared nourishment, it seems more probable that they are degraded forms, perhaps derived from ancestors in which the sexual process was represented. Moreover, the strict inheritance of acquired characters does not occur, since modified species may return to their original characters. Further, it is scarcely possible to eliminate the effects of selective action, which may be the true agent in producing the differences seen in species apparently modified by the environment. Also the power of spore formation under appropriate conditions seems to show that the "germ plasm" and "somatoplasm" of these lowly organisms are not identical.

In view of these considerations, it seems safer to regard the species of bacteria as true species, doubtless variable within certain limits, possibly sometimes polymorphic, but on the whole comparable with species in the higher forms of life. Still the absence of amphimixis must show an effect in a multiplication of races (in contradistinction to varieties) in excess of the number of such forms to be met with higher in the organic scale.

The generic characters are discussed pretty fully by Drs. Helme and Raugé in the article above alluded to. They conclude that the best characters available are the form of the elements and their mode of association; that is, they endorse the present arrangement. As regards motility, they discard it. They say: "ce caractère n'offre ni assez de variété, ni surtout assez de constance, pour apporter dans une nomenclature des données quelque

peu précises." They overlook the fact that though the motility may vary, the flagella may be uniformly present as shown by Moore in the case of *B. coli-communis*,* and further the varying arrangement of these flagella when present, as shown by the classification of Messea.† It appears to the writer, that with a perfectly satisfactory method of staining, the structures upon which motility depends should form the basis of a more nearly natural classification than any in general use. At present, however, the practical difficulties are too great to allow of rapid and accurate work on such a basis.

So far, then, as generic characters go, these are, or may be based upon morphology, as in the higher forms of life. But not so with specific characters. Here morphology fails, for the minor differences in structure, pattern or coloration, etc., which we are accustomed to use, are either lacking, or so slight as to be inappreciable. To separate species, we have to fall back upon physiological characters. In other words, we place the several species under approximately uniform abnormal conditions (artificial cultures) and note their behavior. Perhaps we may be justified in assuming that the several individuals of a species will behave somewhat in the same manner under these definite abnormal conditions, but we have no criterion as to the extent of variation to be expected. Physiological characters have not been used to a sufficient extent among other groups of living things to enable us to predict whether they are more variable than the characters ordinarily used or not. Clearly it is a mistake to assume that they are less variable, though this assumption seems to have been very often tacitly made.

But granting that the physiological characters are sufficiently constant for practical use, we must have enough of them to be able to distinguish positively between every species. The liquefaction of gelatin is not enough. The characters of growth forms and color are often too variable, too similar, indefinite or difficult to describe recognizably. We should have more positive tests, and as such are not wanting they should be universally applied. It is impossible to say how many are needed; this can only be shown by experiment, and when we finally get some idea of the extent of the bacterial flora of the world.

* V. A. Moore, *Wilder Quarter Century Book*, pp. 339-365.

† Messea, *Rivista d'igiene e sanita pubblica*, No. 14, p. 513 (1889).

IDENTIFICATION OF OLD DESCRIPTIONS.

IN regard to insufficiently described species, two courses seem open. We may discard the names of all such and redescribe each as it occurs, or we may apply the name to any species which does not contradict the author's original description and complete the characters from that species. To assist in the recognition of specific descriptions in the higher forms of life, there is usually to be found the original example, or "type," which was before the author and from which it can always be learned what form was intended by the description, however imperfect; when the type is lost, the description, if unrecognizable, is discarded. But in the bacteria, the preservation of types is for the most part, out of the question (since they must be alive), and the former course becomes objectionable, not only in that such a large number of names would have to be discarded, but also that this number would be variable in the opinion of different authors. Further, as time went on, the discovery of new species might render the description of an old one incomplete. According to the second course, every name is to be preserved, the imperfect description being completed by subsequent authors, provided they do not identify as the old species a form which in any way contradicts the original description, and all additions so made are to become a part of the characters of the species. It would appear either that all imperfect descriptions should in future be omitted from our books, or some concerted attempt should be made to complete them. If the latter course be thoroughly applied, and authors can be induced to refrain from describing new species unless no old name can be made to apply, and further, if a new name seems necessary, to show good characters by which the new form can be distinguished from its nearest allies, then we ought in time to obtain a series of characterizations from which it would be easy to determine a given species, and the science of descriptive bacteriology might be placed on a footing of partial equality with other branches of natural science.

It is to be remarked that this branch of the subject has suffered from its close connection with the study of medicine. Not only have the non-pathogenic saprophytes received scant attention, but the eager desire to isolate the active agent in the cause of diseases has led to the naming of many bacteria from very insufficient characters. It would seem that descriptive bacteriology belongs to the

department of Botany,* not to medicine which should rather concern itself with the application of bacteriology to medical subjects.

To summarize then, the present state of bacteriology is this: while some five hundred species of bacteria have been described, it appears that this number is far from covering the entire flora, since apparently new species are met with on every hand. This mass of descriptions is rather a hindrance than a help to further work, for not only are many species imperfectly described as compared with others, but the general standard of specific descriptions is inadequate to give characters to separate with clearness those species already known. Again it is known that species vary, but to what extent is not known. These three factors, then, imperfect knowledge of the flora, incomplete descriptions and ignorance of the extent of variation, tend to render identification of species uncertain, and discourage workers in this field. It is hoped that the present contribution may tend in some small measure to mitigate each of these evils.

VARIATION IN BACTERIA.

Some experiments were undertaken to give an idea of the range of variation in a given species. The results are largely in accordance with the very satisfactory conclusions expressed by Dr. A. Rodet in his recent valuable treatise on variability of bacteria.† Rodet concludes that the several forms of bacteria are generally constant, but that they are to be regarded as different races of comparatively few species. I shall not enter into a discussion of former work on variability, as this has been much better done by Dr. Rodet than I could hope to do within the limits of this paper. I refer to his work.

The following experiments were directed principally toward the point whether the variations among bacteria are generally of the nature of "acquired characters," *i. e.*, due to differences in the environment as seems to have been generally assumed‡ or rather

* Or Zoölogy, if we follow Ernst Haeckel's *Systemat. Phylogenie der Protisten und Pflanzen*, Berlin 1894.

† *De la variabilité dans les microbes au point de vue Morphologique et Physiologique*, par le Dr. A. Rodet (1894).

‡ See Dr. J. G. Adami on the variability of bacteria and the development of races, *Medicine Chronicle*, September, 1892. Also Dr. A. S. Packard on the inheritance of acquired characters, etc., *Proc. American Academy* 1895, pp. 343-344.

"spontaneous variations," occurring in different individuals in the same stock under essentially identical conditions, such as we see among the higher organisms and the mode of occurrence of which has been explained by Weismann as the effect of amphimixis, or, as would apply more exactly to this case, of bud variation by an "abnormal differential nuclear division" (Germ-plasm. p. 442).

Bacillus lactis erythrogenes was selected as the subject of variation experiment. I have shown in a short paper read before the New York Academy of Sciences* that many closely allied and variable forms are to be met with in nature, some of them clearly to be referred to this species, others doubtful. A knowledge of the degree of spontaneous variation of this germ was very desirable in the special relation of determining the standing of these forms, as well as in the general one of the nature of variation in bacteria. It was thought that light might be thrown on both subjects by a study of this species.

SPONTANEOUS VARIATION.

1—*Slight Continuous Variations.*

The variations were tested as to the liquefaction of gelatin, the coagulation of milk, the reduction of nitrate to nitrite, and the amount of pigment produced in an agar culture.

B. lactis erythrogenes commonly produces a quick liquefaction of gelatin; it forms a soft flaky coagulum in milk which on boiling has no consistence and which is gradually dissolved; it reduces the nitrate solution † partially so that the test gives a faint red color, about half way between the deepest tint that can be produced and no color; in other cases completely; in an agar culture, the mass of growth is yellow and a pink tint is seen in the medium.

A culture was selected for experiment, taken from the air, in which the liquefaction of gelatin was rapid (normal), action on milk normal, the pink tint good, the yellow paler than usual, somewhat whitish, and nitrate scarcely reduced at all, in twenty-eight days only showing a faint trace of color with the test. From the culture a series of gelatin plates were made by dilutions. From one of the plates, separate cultures were made in ten tubes,

* Published in the "Transactions" for 1895.

† The formulas for all media used are given at the end of this article.

the first five from surface colonies, the last five from deep ones, and these were all planted at the same time on media exactly alike, and grown on the same shelf under conditions as nearly alike as possible. The following tables exhibit their relative rate of growth. Number 7 failed to grow, probably because no bacilli were actually transferred from the colony, it being a "deep" one.

(The distance of the line to the right in each column indicates the comparative amount of action each culture had produced. For example, in first table on seventh day, No. 2 was farthest advanced and No. 6 least.)

LACTOSE-LITMUS GELATIN.

NUMBER.	2 DAYS.	4 DAYS.	7 DAYS.	10 DAYS.	12 DAYS.	14 DAYS.
1	Slight liquef.	Cup shaped.	Decolorizing.			
2						
3						
4						
5						
6	(Not liq.)					
8						
9						
10						

MILK .21 CC. ACID.*

NUMBER.		4 DAYS.	7 DAYS.	9 DAYS.	11 DAYS.	14 DAYS.
1		(No change.)				
2		(A coagulum.)				
3						
4†						
5						
6						
8						
9						
10						

* Each cc. required .21 cc. tenth normal NaOH to render neutral to phenol-ptalein.

† Not planted at the same time as the others.

MILK .30 CC. ACID.

NUMBER.	4 DAYS.	7 DAYS.	9 DAYS.	12 DAYS.	14 DAYS.
1	No effect.	Not positively.	(No change.)		
2	..	..			
3	..	..			
4*	..	..			
5	..	..			
6	..	..			
8	..	..			
9	..	..			
10	..	..			

REDUCTION OF NITRATE.

NUMBER.	6 DAYS.	13 DAYS.	20 DAYS.	28 DAYS.
1	(No action.)		(Faint trace)	
2				
3				
4				
5				
6				
8				(Not reduced.)
9				
10			(Faint.)	

As the rate of action of the culture on its medium depends a good deal on the "dose," or the number of bacteria introduced on the inoculation needle, the effect of this factor must be allowed for. There is no way of regulating this adequately; but I think the above table shows that there is individual variation in the descendents of a single cell, apparently independently of the environment. The number of the cultures practically eliminates the effect of the dose, when we take an average of them. It will be noticed that on the whole No. 10 produces the most marked effect, and No. 8 the least.

I find that in taking cultures in the ordinary manner from one tube to a new one by taking up a mass of growth on the needle,

* Not planted at the same time as the others.

the resulting cultures tend to exhibit the same characters as the parent culture. When, however, cultures are made from separate colonies from a plate, the individual variations tend to become much more apparent, as the above shows.

To proceed with the experiments. Having obtained cultures derived from a single one but with slightly different characters, I proceeded to test these further, with the application of selection, as follows :

(1) No. 6 was selected as showing the least power of liquefying gelatin and a series of plates were made from it by dilution. Of the resulting colonies, nine were planted on gelatin and of these nine I selected the one which liquefied gelatin most rapidly and the one which liquefied the least so (for there was a considerable difference, as in the first instance) and planted them side by side with the original No. 6. Here is the result in tabular form as before :

	2 DAYS.	4 DAYS.	6 DAYS.	9 DAYS.	12 DAYS.	14 DAYS.
Number 6, . . .						
New 3 (best),						
New 8 (poor- est),						

It shows that instead of holding true to the character acquired by No. 6 of a slower rate of liquefaction, there was a marked tendency to return to the original quick liquefaction, which could probably alone be overcome by a long course of selection. Notice also the difference in the rate of growth as shown by the fact that the No. 6 finally catches up with the new No. 8, perhaps owing to the approaching exhaustion of the medium.

(2) No. 10 was selected as showing the best effect of reduction of nitrate, being much better than the original culture. It was hoped, by selection, to produce a form which would reduce nitrate as well as normal *B. lactis erythrogenes*. Notice that there was at first a marked tendency to approach the normal type of *B. lactis erythrogenes*. The original culture corresponded in its reducing effect to No. 8 above, whereas eight out of nine of its progeny reduced nitrate better than it did. It was thought that this function might be easily further increased.

Ten colonies were tubed from gelatin plates made from No. 10, and planted in nitrate solution at the same time. Of these it was

found that only three retained the same degree of power of reduction, four fell back to the average of the table given above, while three returned to the condition of the original culture. Here we see again the marked tendency to return to the type, even in this slightly differing race. These results indicate the comparative permanency of the species and also the races of bacteria as stated by Rodet to be the case.

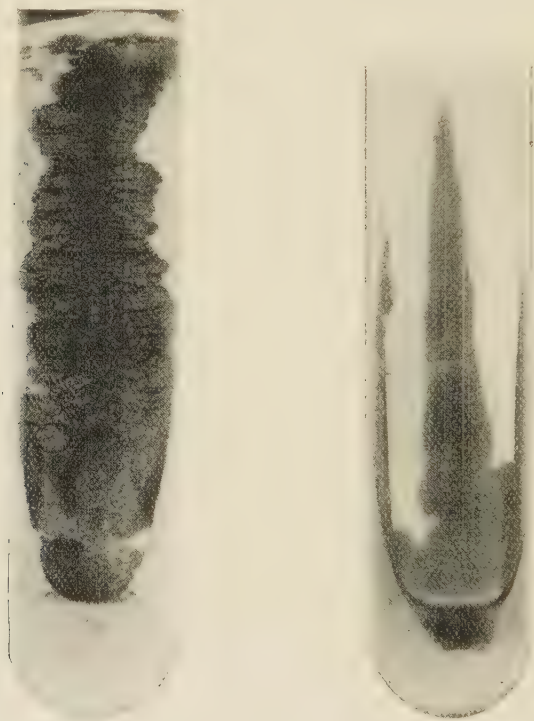
(2) *Sports or discontinuous variations.*

In the discussion of this subject, the existence of uniform conditions is presupposed. I consider that when the tubes are filled with media from the same flask, are planted at the same time, and grown on the same shelf, that these conditions are fulfilled. Or even the plantings need not be made at the same time, provided the conditions of temperature are approximately uniform. We see cultures taken from both deep and surface colonies, from different media, etc., exhibit the same characters when transferred to the test media. Now if one of these suddenly exhibits different characters, it is absurd to attribute the change to the action of some slight, unknown and undemonstrable difference in the medium or other condition. The variation is spontaneous and due to the inherent properties of the germ.

Among the Protista, one objection is always forthcoming against the evidence of discontinuous variation, which does not trouble us higher in the organic scale. It can always be said that the variety may be a contamination, and it is almost impossible to meet this objection completely. Nevertheless, I shall attempt to meet it in the following instance, at least to a considerable degree.

In the species under investigation, *Bacillus lactis erythrogenes*, the growth on solid media is smooth, thick and soft, uniformly light yellow, the growth quite softly granular to the needle, not in the least crusty or viscid. A culture sent to me from Král's laboratory (marked *B. helvolus* Zimmerman) became contaminated during the summer vacation with a motile spore forming bacillus (*B. mesentericus vulgatus*). In the process of purifying it, gelatin plates were made and a culture from one of the yellow colonies developed a somewhat coarsely granular growth. It was set down as a contamination, although the fact that it produced a slight pink tint in the medium and reacted on all the media nor-

mally for *B. lactis erythrogenes* made me suspect that it might be a variety. Later in the course of the investigation on nitrate reduction on a culture obtained from the air of New York, one of the nitrate tubes showed a growth a little abnormal, the precipitate being slightly flocculent, whereas it is usually finely granular.



B. ERYTHROGENES RUGATUS.

B. LACTIS ERYTHROGENES.

This nitrate culture was one of ten made from an agar plate of the culture No. 10 as described above. A culture on agar gave a flattened, yellow growth, covered with coarse wrinkles and entirely unlike *B. lactis erythrogenes*. (See figure.) Still all the biological characters were normal, even the peculiar effect on milk, but there

was not a very strong pink tint in the medium. Other agar cultures repeated the characters. Colonies on agar plates were moderately brittle, the surface ones large, flat, and marked with coarse granules centrally, and a rim-like margin. Morphology normal. In short, this form corresponded in all respects with the culture from which it was apparently derived, except in the peculiar alteration in the consistence of the growth mass. But the difference is one that would be usually considered specific.* Now the usual precautions to avoid contamination were observed here and it is rather unlikely that this culture was a contamination, especially considering the improbability that a contamination should reproduce all the morphological and biological characters so exactly. Moreover, I have seen no such species as this would have to be considered. In spite of these considerations, I am unable to make the assertion that we have not to do with a contamination, but the following experiment occurred to me to test the matter further. I have shown above that there is a tendency for a variety to return to the parent form. Therefore, if this be a variety, some of the colonies should show a return to normal *B. lactis erythrogenes*. If, however, it be a different species accidentally introduced into the original gelatin plate, we should not expect to see any such result. Some agar plates were consequently made and on the third dilution there resulted one hundred and twenty-five large surface colonies, besides many deep ones. Of these one hundred and twenty-five, three showed the smooth surface and soft texture of *B. lactis erythrogenes*. The others were all alike, granular centrally, with a rim-like margin, and liable to crack in two under the needle. Cultures from the former resulted in a normal growth of *B. lactis erythrogenes*, the others reproduced the wrinkly growth. Now there are but two alternatives; either this is a discontinuous variation, which occurs in this species, and which I have thus happened upon in two instances, possessing a certain tendency to return to the type, or there is a species closely allied to *B. lactis erythrogenes*, but differing in texture of growth, which shows a marked tendency to contaminate cultures of this species (!), but is not otherwise common. In my last experiments this "species" must have entered

* Dr. Prudden has called my attention to the fact that a very similar difference in growth form exists between the *Bacillus tuberculosis* and the *B. tuberculosis gallinarum*.

my nitrate culture, but not entirely overpowered the *B. lactis erythrogenes*, so that both could appear in the plate in the proportions described.

To eliminate this last alternative, plates were again made, this time from a growth taken from one of the rough surfaced colonies just described. This culture has now been through the usual process of purification, and ought not to be contaminated. It exhibited the characteristic wrinkled surface of the form under investigation. Now it is to be remarked that even if no reversion were demonstrable, it would not prove that this form was originally a contamination, for the original tendency to reversion being three out of one hundred and twenty-five, I might have practically eliminated it by this selection. On the other hand, if reversion occurred, it would be evidence in favor of this form being a discontinuous variation. The plates were made with the following result: One third-dilution plate gave about one hundred and fifty surface colonies, all of the wrinkly form; a second third-dilution plate gave about one hundred and forty colonies, three of which were of the normal soft form. Here again the reversion showed itself, but with a distinct tendency to diminution; three out of two hundred and ninety.* These wrinkly colonies did not reproduce the form of colony described above; they were more folded and wrinkled with uneven edge; but the growth on agar tubes was not noticeably different. In a second "generation" agar culture made from one of these tube cultures when it had become old, a narrow border of the soft growth appeared around the edge, suggesting that either reversion had occurred in the culture itself or that the latter was not pure. Consequently plates were again prepared. The resulting colonies were obtained only few on a plate (about fifty-five surface ones) so as to give them plenty of room. Of these colonies none developed the normal soft form, but when they had become old some exhibited the bordering soft growth just as in the tube from which they were taken, but the border was present in varying degrees. Four cultures were made from different appearing colonies as follows: 1, from a colony all wrinkly, resulting growth all of the wrinkly form; 2, from a colony before definite characters had ap-

* Notice the much less tendency to reversion in the discontinuous variation than among the slight continuous ones. Is this a general character of these classes of variation?

peared, resulting growth wrinkly with a considerable soft edge; 3, from a deep colony (characters therefore not known), resulting growth about half wrinkly, half soft, mixed; 4, from the smooth border of a colony, resulting growth all of the normal soft form. These results are equally well explicable as the results of contamination as of reversion.

Next plates were prepared from tube No. 1, just described. As no soft border was detected, the growth was presumably, but not positively, of the pure wrinkly form. The resulting colonies were obtained twenty-five on two plates, and consequently well separated. Most of them were of the very wrinkly form with uneven contour and folded surface, and several of these exhibited the soft border in varying degrees. In some it was all around; in others, on one side only, and in one the colony was half soft, half wrinkly. About a quarter returned to the original form of colony with granular surface and rim-shaped margin, and these had no soft borders. No pure normal, soft colonies were seen. The agar tubes made from the very wrinkly colonies and the rimmed-margin ones were scarcely to be distinguished at a casual examination; but a closer observation showed slight differences of the same nature as those between the two forms of colonies. The difference is so slight, however, and so soon becomes obscured by the advance in growth, that I did not notice it previously in the investigation, though it must evidently have obtained.

At this stage of affairs, we have presented the following alternatives: either the variations which I am trying to establish or disprove do occur, or the following condition is operative. It is conceivable that when the skinny growth of the wrinkly form is agitated in sterilized water preparatory to making plates, that on account of its property of coherence, instead of becoming separated into individual cells, certain of which are subsequently to develop colonies, the smallest portions really consist of masses of cells which might entangle a few cells of the smooth form, if this were originally present as a contamination. Thus the resulting colonies might be impure and the apparent phenomena of reversion be due to a separation of this mixture. Now the phenomena described above, of the soft borders to the wrinkly agar cultures and colonies, are in favor of this view, and it was also observed that the growth when shaken up in water disintegrated with great difficulty. On the other hand, the existence at this time of

three different forms, differing only in manner of growth and occurring so sporadically, is much against the contamination theory. Thus it becomes evident that this line of research is not competent to positively exclude the possibility of contamination, though it has rendered it a complicated explanation. It was abandoned at this point.

Now, on the other hand, in making plates of the soft form, the difficulty of the cells possibly adhering in masses is reduced to a minimum. A softer growth is scarcely to be imagined than that of the normal *B. lactis erythrogenes*. In the hanging drop, the cells are seen singly, or, rarely, in pairs or short chains. It is therefore, highly improbable that these should form masses and entangle a few of the wrinkly form. The worst that might be expected would be that the soft and wrinkly forms should adhere in approximately equal numbers, and the resulting colonies could not be mistaken for pure colonies of the soft form. Moreover, whereas a slight growth of the soft form might escape detection in the growth mass of the wrinkly form, it seems almost certain that in the reverse condition the mixture would be easily detected. Taking advantage of these conditions, the question was approached from this side. An agar culture was made from a soft colony occurring on the first "reversion plate" made from the original wrinkly culture. It appeared to be a pure culture of normal *B. lactis erythrogenes*. A pair of third dilution plates prepared from it exhibited about 140 coloneis, all of the normal soft form.* A second pair of plates exhibited 340 colonies, and of these one had returned to the wrinkly form, and gave a typical wrinkly growth on agar; twelve exhibited a new variety, not previously met with, and the rest were of the normal soft form. The new variety possessed a slightly irregular surface, but was soft to the needle. Its growth masses on agar were slightly wrinkly, with the outline finely marked just before the edge as if milled, and altogether were intermediate between the wrinkly form and the normal soft form, but nothing like a mixture of the two. Thus it would seem to be established that these various growths are discontinuous varieties of *B. lactis erythrogenes*,

* There occurred also on these plates some colonies of *Bacillus ramosus* derived from the sterilized water used in making the plates. The water was boiled just before using as an extra precaution, but the resistant spores of this species were not destroyed.

barring the possibility of some method of contamination which I have not thought to guard against.*

Summary of the Variations of Bacillus lactis erythrogenes.

Form 1. (Normal) Soft, smooth growth in colonies and in tube.

Form 2. Soft and smooth, but the colonies with lobed edges; growth in tube finely creased and milled before the edges.

Form 3. Rather crusty, the colonies even, flat, granular centrally with a well defined rim-like edge; in tubes a flat wrinkled and folded growth with irregular edges.

Form 4. Slightly more skinny than form 3. Colonies and growth on tube alike, very much folded, with irregular edges, tending to produce a margin of the soft form. (See the figure; also the description of No. 107, *B. erythrogenes rugatus*.)

Form 5. There is also to be recorded here the granular variety derived from *B. helvolus*. (See No. 106, *B. helvolus granulatus*.)

Note—These are only variations in the form of growth. All the cultures were of the shade of yellow of the particular form of *Bacillus lactis erythrogenes* from which they were derived, and produced also the pink tint. So far as tested, they reproduced the biological characters of their parent culture, which, as previously noted, were not in all respects normal.

EFFECT OF THE ENVIRONMENT.

As an example of the effect of the environment, the action of a change of temperature was selected. It is well known that many chromogenic species, when grown "for several generations" at the body temperature, produce white races, which are more or less permanent. What is the nature of this process?

The same rather pale yellow culture of *Bacillus lactis erythrogenes* was subjected to experiment. The culture No. 6 exhibited a pale growth on agar, especially whitish along the edge. A culture from this edge kept at $37\frac{1}{2}^{\circ}$ C. for two days, and then transferred to the room temperature, grew nearly white. Three subsequent "generations" treated in the same manner produced

* Two more sets of plates were made from the last obtained smooth growth, without further reversion; all the colonies were alike. But, as the number of colonies in the two platings was 525 and 1685 respectively, whereas the tendency to reversion was calculated to have diminished at least to 1:950 and 1:1900 respectively, the results are not conclusive against the variation hypothesis.

no further whitening of the growth. It was noticeable that each culture grew less vigorously than the preceding while in the incubator, though it seemed to revive fully when removed to the room temperature. Culture No. 1 was of a distinctly more yellowish shade than No. 6, and not whitish at the edge of the growth. A culture made from it, grown two days in the incubator, and transferred to the room temperature, gave a paler yellow; one from this, about the same tint; the next generation was less uniform, somewhat spotted with white and yellow, and the next generation was distinctly spotted. The same debilitating effects of the repeated exposure to the high temperature was noted here as with No. 6. The last, most spotted (*i. e.*, least yellow) culture was examined as follows:

(1) It was allowed to grow well for several days at the room temperature. It was then seen that the yellow parts of the growth were less active in extending their borders than the white parts were, which resulted in an uneven outline of the growth mass, the yellow fans terminating at the incisions. (2) A set of agar plates was made from this growth. Of the resulting colonies, some were nearly white, others distinctly yellow. It was noted that, on the whole, the yellow colonies were smaller. None of the largest were yellow, though some of the yellow were as large as some of the white ones. (3) Cultures were made from a white and a yellow colony respectively, from one of these plates, and they were planted on milk, nitrate solution and lactose-litmus agar. On all these media the white culture advanced more rapidly than the yellow one; the gelatin was slightly but decidedly more quickly liquefied, the milk more quickly coagulated and the nitrate more strongly reduced in the same time and under identical conditions.

From the above I conclude that the white form is less injuriously affected by the abnormal condition of increased temperature than the yellow form is, and the apparent effect of the temperature in producing a white form in the first instance may have really been due to a process of selection, the white form growing the faster and tending to supplant the other. Thus the ordinary course of transference of a small part of the growth on the inoculation needle would be the agent in this selection. Now it is a fact that the different cultures of *B. lactis erythrogenes* obtained from different sources vary a good deal in tint, and I have indi-

cated that there was some spontaneous variation in this respect in my experimental cultures. The variations toward a white form probably tend to become eliminated under natural conditions where the yellow form doubtless grows best,* but under the effect of higher temperature there comes room for the action of the selection which I have just described. It may be that this is, at least in great part, the true explanation of the production of the colorless races of chromogenic bacteria, which has been quoted as proof of the "transmission of acquired characters."

EFFECTS OF DIFFERENCES IN THE COMPOSITION OF THE MEDIA.

I need not enter into a long discussion of this subject. It is well known that slight variations in the composition of the media make marked changes in the bacterial growth. I have shown elsewhere† the effect of varying degrees of acidity in gelatin; the difference in the action of my selected cultures on the two samples of milk from different sources and with different degrees of acidity, which is tabulated above, is a case in point; again it was noticed that the chromogenic power of some species was less on my media made with meat extract than it was in the same ones grown on media in which the meat itself had been employed.

These changes are not true variations, as they are not inherited but I have given the exact composition of all the media used in this investigation, to eliminate this source of error. The receipts will be found at the end of the article.

CONCLUSIONS AS TO VARIABILITY.

From the above set of experiments I infer that it is premature to assume that races of bacteria are produced by the direct action of the environment. Rather the species possess, first a power of continuous variation, producing intergrading varieties, and which under a long process of natural selection is capable of adapting them to various situations or functions; second, a power of considerable discontinuous variation, producing "sports," dimorphic or polymorphic forms or races (which may revert to type spontaneously; but which are distinguishable from true species only

*In some old cultures of both the white and yellow forms, which had become partially dried, a few lumps of vigorous growth started out, and these were of the yellow form.

† See Transactions of the New York Academy of Sciences, 1895.

by the occurrence of such revision) and ultimately species, by the lapsing of the capability of reversion.

In the following systematic account, I have provisionally adopted the more conservative course of giving all differing forms specific rank until their exact relations be determined. Many of them will doubtless come to be regarded as races or varieties; some might properly be so placed now.* Those forms which do not differ except in degree and to no great extent are considered conspecific.

Unfortunately, we do not seem to have arrived at the point where it is possible to differentiate readily species from races and varieties. This results from the fact that there is no standard by which to determine which characters are more reliable. If the several forms be divided on a single character; for example, if we select all yellow species, or all which reduce nitrate or liquefy gelatin, etc., the grouping is different in every instance. There is no indication of the formation of reliable groups by a convergence of the several characters, so that the classification is entirely arbitrary, depending upon which character is given prominence. Consequently, I have as yet found no characters to form a series of natural groups of species under the genus which has been urged on me by Dr. Prudden to be very desirable.

In this paper, the term "groups" has been used in a specific rather than in a subgeneric sense; for instance, the "anthrax group" means a series of forms which are probably varieties of one species; but, as I have given all varietal forms the specific position, I have preferred to use the term as I have, with this explanation.

A LIST OF THE COMMON BACTERIA OF THE AIR OF NEW YORK CITY.

The following list embraces all species found more than once in the air in the course of this investigation. Those occurring but a single time will be referred to in the systematic part. The cultures were obtained by exposing gelatin plates for from one to five minutes in various situations. Of the resulting colonies, a portion only were transferred to tube cultures for investigation. The yeasts, Cladothrices, and moulds were disregarded. In numbers the Micrococci were considerably predominant, but of comparatively few species. The Bacilli, though less common, were

* For the sake of uniformity this has not been done.

more often of different species, the commonest class being the very short forms, "Microbacilli." No Spirilla were found. Twenty-four Micrococci and forty-four Bacilli were found in the air, and of these the following nine Micrococci and nine Bacilli occurred more than once:

<i>Micrococcus concentricus</i> , Zimm.	<i>Bacillus fuscus</i> , Zimm.
<i>Micrococcus cremoides</i> , Zimm.	<i>Bacillus finitimus ruber</i> , Dyar.
<i>Micrococcus cremoides albus</i> , Dyar.	<i>Bacillus decolorans major</i> , Dyar.
<i>Micrococcus pyogenes aureus</i> , Passet.	<i>Bacillus candicans</i> , Frankland.
<i>Sarcina flava</i> , DeBary.	<i>Bacillus inutilis</i> , Dyar.
<i>Micrococcus mobilis</i> , Maurea.	<i>Bacillus Hudsonii</i> , Dyar.
<i>Merismopedia rosea</i> , Bumm.	Flügge.
<i>Micrococcus tetragenus vividus</i> , Dyar.	<i>Bacillus lactis erythrogenes</i> , Hueppe.
<i>Micrococcus tetragenus versatilis</i> , Stern.	<i>Bacillus helvolus</i> , Zimm.

SYSTEMATIC ACCOUNT.

I will proceed to detail the morphological and biological characters of all the species which I have worked out in the course of this investigation.* I have prepared synoptic tables, so that all of these species can be readily and quickly identified. The composition of all the media of cultivation made use of is given at the end of the systematic account. As far as possible, previously used media have been employed, and the most often used of these have been given prominence (though they do not always deserve it from the point of view of reliability) in order to bring in line the work already recorded by others in description of species. My work has mainly consisted in the general application of a series of tests uniformly to a rather large group of species, and the result in the manner of their separation seems, on the whole satisfactory. If this work could be extended to cover all the species of Bacteria, I think the operation of identifying species would be far less confusing than it is at present. An objection which I have not been able to overcome is the great length of

* This includes all those planted for comparison as well as those derived from the air.

time necessary to determine a species. I have felt obliged to use the action on gelatin as the primary character, because it has been the only medium in general use; but I find it necessary to grow cultures at least two months before becoming certain that liquefaction will not ultimately ensue. To the other media, I have set the limit of twenty-eight days. All cultures were grown at the room temperature, unless otherwise stated.

In the Mircococci I have used the terms Merismopedia and Sarcina in the generic form to indicate the general mode of association of the elements; but I do not wish them to be understood as of true generic value. They are, in some instances, of less than specific value; but they are convenient terms, and I have used them as such. It is to be hoped that further study will develop some reliable practical morphological characters which will enable us to divide the two unwieldy genera, *Micrococcus* and *Bacillus*, in a satisfactory manner. The mode of association of the elements is not such a character,* the opinions of Drs. Helme and Raugé to the contrary notwithstanding.

In several instances I have not been able to differentiate species occurring in the air and presumably saprophytic, from pathogenic parasites, and I am led to the conclusion that the pathogenic property is not in all cases a test of specific difference, but rather a varietal or racial character. The bearing of this conclusion on the classification adopted in Eisenberg's "*Bakteriologische Diagnostik*" should be noted.

The characters used in the table will be self-explanatory for

* The mode of association of the elements is not a satisfactory generic character, because the differences between *Micrococcus*, *Diplococcus*, *Merismopedia* and *Sarcina* may be due solely to differences in the degree of the coherence of the cells. They may also arise from actual differences in the manner of multiplication, it is true, as follows:

(1) Cells dividing in only one plane, forming *Micrococcus*, *Diplococcus*, *Streptococcus*,

(2) Cells dividing in two planes, forming *Micrococcus*, *Diplococcus*, *Merismopedia*.

(3) Cells dividing in three planes, forming *Micrococcus*, *Diplococcus*, *Merismopedia* and *Sarcina*.

But this theoretical difference remains to be demonstrated, I think, between the last two, and it is certainly so obscure a one in practice as to warrant the above remarks. At best these characters give but three genera instead of five or six. *Streptococcus* is not included in this paper, and my species may be all *Sarcinæ* with a greater or less tendency to break up into single cells.

the most part when the media used are taken into account. Gelatin is to be regarded as liquefied even when the fluid evaporates as fast as formed and only a dry hollow results. Consequently, if any hollow is formed by the growth mass, the gelatin is to be considered liquefied.

In the descriptions I have not repeated the characters given in the synoptic table except where necessary to enlarge on them.

THE CHARACTERS FOR WHICH THE SEVERAL MEDIA ARE USED.

It is important that the number of media should not be unnecessarily multiplied, or else the labor of determining species will become too great. The following shows the characters which I have obtained from the several media used, and how labor may be saved in some instances :

Broth. This medium may usually be omitted. The characters to be obtained from it are good ones, but can usually be detected as well in the fermentation tube and water of condensation in the agar cultures.

Gelatin. Only used for the liquefaction test and the characters of colonies on plate. I have found it practicable to dispense with a separate planting on simple gelatin by preparing a lactose-litmus gelatin, which combines the characters of liquefaction and acid or alkali formation in one medium. I have disregarded the decolorizing effect on litmus so often seen, as I have not found the characters reliable on solid media.

Agar. Used for the growth forms and necessary. Glycerine agar is less important, and I have not used it for diagnostic purposes.

Fermentation broth in bent tubes is necessary for the gas formation test and relation to air. The gas formation is often indicated on lactose-litmus gelatin, and in such cases scarcely needs confirmation on this medium.

Milk for the effects of coagulation. It should always be boiled before the test can be considered complete.

Nitrate solution. The test for nitrates may be applied by adding two to five drops of naphthylamine sulphate, a small crystal of sodium sulphanilate and boiling the medium to hasten the reaction. When cool it is ready to examine for the color. I do not test for ammonia.

Rosolic acid broth. Useful for decolorization effects. The deepening of the tint is of little value.

Pepton and salt broth. For the formation of indol. The test may be applied by adding a few drops of sulphuric acid, and then a very dilute sodium nitrite. Care should be exercised not to add too much. This test is open to the same objection as is the nitrate test, and in greater degree, in that the characters to be obtained from it are not sharp ones.

BACTERIOLOGICAL DIAGNOSIS.

Species which will grow on the ordinary media, in presence of air at 20° C.

MICROCOCCI.

The gelatin is not liquefied	page 345
The gelatin is ultimately liquefied	page 348

BACILLI.

No gas formed in the fermentation tube.	
The gelatin is not liquefied	page 355
The gelatin is ultimately liquefied.....	page 364
Gas is formed in the fermentation tube	page 375

SPIRILLA (not found in the air).

Species which are anaerobic, will not grow on the ordinary media, or require a higher temperature than 20° C. (not investigated).

NON-LIQUEFYING MICROCOCCI.

(Synopsis of species).

Lactose-litmus gelatin reddened.

Chromogenic.

Yellow ; nitrate strongly reduced	1
Red ; nitrate very slightly reduced	2

Not chromogenic.

Milk coagulated.

Nitrate slightly reduced; milk coagulated.....	3
Nitrate not reduced	4

Milk not coagulated.

Growth on agar opaque white.....	5
Growth on agar thin, transparent, poor	6

Lactose-litmus gelatin made blue.

Nitrate not reduced; milk not coagulated.

Rosolic acid faded.....	7
-------------------------	---

Rosolic acid not changed

Chromogenic; orange	8
Not chromogenic; white.....	9

Nitrate reduced; milk not coagulated.

Not chromogenic; white	10
Chromogenic; red	11

1. **Merismopedia flava varians** n. sp.

Occurrence. Abundantly in a jar of "sterilized milk" bought at a store; with no. 101.

Morphology. Micrococci about 1μ in diameter, in twos, fours or in elliptical pairs with a cross-furrow.

Biology. Gelatin not liquified in 60 days; milk coagulated on boiling in 6 days, more evidently later, but not without boiling; nitrate quickly and strongly reduced even in 7 days; lactose-litmus quickly reddened, but in 50 to 60 days it becomes blue at the upper part of the culture; aerobic. In broth there is a fine turbidity and considerably granular yellow sediment; on the solid media a broad pale yellow layer is developed, which may vary in shade, even in different parts of the same culture; on glycerin-agar and potato the growth is abundant, shining bright yellow, opaque. In gelatin plates the surface colonies are much larger than the deep ones, opaque, scarcely shining, light yellow, the edges a little wavy; the deep colonies are round, opaque, yellow, inclined to be slightly irregular.

2. **Merismopedia cinnabareus** (Flügge).

Occurrence. In the air of the College hallway.

Morphology. Micrococci about 1μ in diameter, in twos, fours or in elliptical pairs with a cross-furrow.

Biology. Milk is not coagulated at first, but in twenty-one days a soft cheese is formed which boils up into a very fine coagulum scarcely perceptible.

Nitrate is only very slightly reduced in twenty-eight days. Rosalic acid is not changed. On agar, the growth is not abundant, brownish red, almost orange. Gelatin colonies are all alike, round, orange colored.

Remarks. I may be mistaken in identifying this as Flügge's species (*Micrococcus cinnabareus*).

3. **Micrococcus candicans** (Flügge).

Occurrence. A culture from Krål's laboratory.

Morphology. Micrococci $1-1.25\mu$ in diameter, singly, in pairs or groups.

Biology. Milk was not coagulated when the culture was first received. A year later a coagulation was formed on boiling at the end of twenty-six days; again a solid curd was formed in this time. Nitrate is slightly reduced, the test giving a faint color in twenty-eight days. The growth is white and opaque on solid media, no surface growth in liquid media. Surface colonies are thin, the deep ones round and opaque, white. Rosolic acid is not changed.

4. **Micrococcus rosettaceus** (Zimmermann).

Occurrence. "Micrococcus aurantiacus" from Krål's laboratory.

Morphology. Micrococci $.7-.8\mu$ in diameter, associated in masses.

Biology. Milk is coagulated (on boiling) in fourteen days, and does not change up to twenty-eight days. Growth on solid media is shining white, opaque, soft; no surface growth on broth. Rosolic acid unchanged.

Remarks. This is nearest to *M. rosettaceus*, though perhaps not identical.

5. **Merismopedia tetragenus** (Gaffky).

Occurrence. "Micrococcus tetragenus" from Krål's laboratory.

Morphology. Micrococci .7-.8 μ in diameter, in pairs, a few fours and irregular groups.

Biology. Besides the character given in the synopsis, rosolic acid is not changed; on solid media the growth is opaque white; no surface growth on broth.

6. **Micrococcus similis** n. sp.

Occurrence. In the air of the college hallway.

Morphology. Large cocci, never quite spherical, and frequently divided by a constriction to one side of the middle. Very variable, diameter 1-2 μ . The cells are larger than the *Bacillus diphtheriae* and decidedly rounder, more like cocci.

Biology. Grows very poorly at all temperatures. On agar the growth is obscure, narrow, translucent whitish. On the lactose-litmus agar there is faint growth with a slight reddening of the medium; rosolic acid is not faded. The gelatin colonies are round, opaque white, uncharacteristic. There is little reduction of nitrate, especially marked 37½° where a distinct test was obtained in twenty days.

7. **Micrococcus concentricus** (Zimmerman).

Occurrence. (1) "M. concentricus" from Krål's laboratory. (2) Eighteen different times in the air of the college hallway.

Morphology. Micrococci .6-1.2 μ in diameter, singly, in twos, in short irregular chains or in masses.

Biology. Well distinguished by its power of decolorizing rosolic acid. The growth on solid media is rather translucent white, sometimes concentrically marked. On liquid media the growth is soft and somewhat stringy, often forming around the surface on the glass. Growth on potato abundant, shining sordid white.

8. **Micrococcus cereus aureus** n. sp. ?

Occurrence. (1) "Staphylococcus* cereus aureus" from Krål's laboratory.

(2) A contamination from the air of the laboratory.

Morphology. Cocci .7-.8 μ in diameter, singly, in pairs, threes or masses.

Biology. On agar and potato shining bright orange. In broth a heavy orange colored sediment with no surface growth.

Remarks. I have seen no description with the above name, under which the culture was received. This is only a variety of the following species, which is the non-chromogenic form.

9. **Micrococcus cereus albus** (Passet).

Occurrence. (1) "Staphylococcus cereus albus" from Krål's laboratory. (2) In the air on 59th St.

*Of all the generic terms proposed for the various associations of the cocci, probably "Staphylococcus" is the least valuable. I would definitely discard it.

Morphology. Micrococci 1-1.5 μ in diameter, often seen constricted for division, singly or irregularly grouped.

Biology. The growth on solid media is white, not very opaque; a white sediment in broth; rosolic acid not changed. The gelatin colonies are round, uncharacteristic, the deep and surface ones much alike.

10. *Merismopedia ceriviseae* (Baleke).

Occurrence. (1) "Sarcina flava" from Král's laboratory. (2) In the air of the college hallway.

Morphology. Micrococci .7-1.5 μ in diameter, singly, in pairs, rarely in fours, in masses.

Biology. Nitrate is strongly reduced, the full deep color of the test being shown in from six to fourteen days; rosolic acid unchanged. In broth, a granular precipitate. On solid media the growth is white or very faintly yellowish (2), moderately opaque.

Remarks. These cultures seem not to be contradicted by the description of *Pediococcus ceriviseae*.

11. *Merismopedia havaniensis* (Sternberg).

Occurrence. (1) From the college collection. (2) In the air of the college yard near 59th St.

Morphology. Micrococci, slightly elliptical, often with cross furrows. .7-1 μ in diameter, in twos, fours, or breaking apart into irregular chains.

Biology. Nitrate is slightly reduced, never strongly; growth on solid media is red and a fine red surface growth occurs on milk without affecting the medium perceptibly; a granular precipitate in broth.

Remarks. This is the *Bacillus havaniensis* of Sternberg; but I have preferred to associate it with the Micrococci on account of the grouping of the elements.

LIQUEFYING MICROCOCCI.

(Synopsis of species).

Lactose-litmus reddened.

Milk coagulated; nitrate not reduced.

Large Micrococci, growth on agar lobed.

Growth orange colored 12

Growth cream colored 13

Growth white 14

Smaller Micrococci, growth normal.

Associated in masses.

Growth orange 15

Growth white.

Quickly liquefying 16

Slowly and imperfectly liquefying 17

Associated in pairs and fours 18

Milk not coagulated.

Nitrate not reduced.

Growth yellow.	
Large Micrococci	19
Minute Micrococci	20
Growth pinkish thin	21
Nitrate reduced	22
Lactose-litmus not changed or made blue.	
Milk coagulated.	
Growth orange	23
Growth yellow	24
Growth white	25
Milk not coagulated.	
Motile.	
Orange	26
Pink	27
Not motile.	
Orange	28
Pink	29
Yellow.	
Growth granular, a normal precipitate in broth.	
Bright lemon yellow	30
Paler yellow	31
Pale creamy yellow	32
Growth forming surface skin, wrinkly	33

12. *Micrococcus cremoides aureus* n. sp.

Occurrence. Obtained by Dr. Freeman from the air of a barn where a cow was being milked.

Morphology. Micrococci 1-1.25 μ in diameter, associated irregularly.

Biology. Gelatin is rapidly liquefied; the surface colonies on gelatin plates are somewhat peculiar; in the cups of liquefaction which are quickly formed, masses of opaque orange flocculi settle in a ring shape about a clear central area, giving the appearance of an indented margin. Liquefaction sets in much later with the deep colonies. They appear spherical, light yellow and opaque. Milk is quickly coagulated, forming a solid curd which is slowly dissolved. Rosolic acid not changed. Lactose-litmus is quickly reddened. The growth on agar is narrow, shining, the edges uneven, finely lobed, orange color. The lobed edges are rather characteristic. On glycerine agar the growth is scarcely chromogenic, being very nearly white, but the normal color is regained when transferred to ordinary agar. At 37½° C. also the growth is not chromogenic. In broth there is a slight surface growth beside a pale orange sediment.

Remarks. This species is to be regarded as a varietal form of the following:

13. *Micrococcus cremoides* (Zimmermann).

Occurrence. (1) In the air of the college hallway. (2) In the air on 59th St.

Morphology and biology as in No. 12, but the growth cream-colored instead of orange. On glycerine agar and at 37½° C., the growth is white.

Remarks. This seems to correspond with *M. cremoides* of Zimmermann, except that the cells are larger. *Cremoides* is said to measure $.8\mu$, while the present species measures $1-1.5\mu$.

14. *Micrococcus cremoides albus* n. sp.

Occurrence. (1) Twice in the air of the college hallway. (2) Twice in the air of the yard near 59th St.

Morphology and biology as above. (Nos. 12 and 13), but the growth pure white on all media.

Remarks. This is a white form of the preceding.

15. *Micrococcus pyogenes aureus* (Passet).

Occurrence. (1) "*Staphylococcus pyogenes aureus*" from the college collection. (2) Twice in the air of the yard near 59th St.

Morphology. Micrococci $.7-1\mu$ in diameter, associated in irregular masses.

Biology. Gelatin quickly liquefied; milk coagulated, forming a hard curd which is slowly dissolved; nitrate not or very slightly reduced. Growth on solid media orange, the edges of the growth even. In gelatin plates the colonies sink in cups of liquefaction without any peculiar appearance. Culture (1) was chromogenic on glycerine agar and at $37\frac{1}{2}^{\circ}\text{C}$; the one first obtained from the air was chromogenic at $37\frac{1}{2}^{\circ}\text{C}$, but grew nearly white on glycerine agar and the third culture grew white in both these cases. Rosolic acid not changed.

16. *Micrococcus pyogenes albus* (Passet).

Occurrence. In the air of the college hallway.

Morphology and biology as above, but the growth is white, on agar rather translucent.

Remarks. This is probably to be regarded as not specifically distinct from the preceding.

17. *Micrococcus epidermidis albus* (Welch).

Occurrence. (1) "*Staphylococcus epidermidis albus*" from the college collection. (2) "*Micrococcus* of Freire" from the college collection. (3) In the air on 59th St.

Morphology and biology as above, but gelatin is very imperfectly liquefied. The liquefaction begins in about six days, but proceeds very slowly, and never far; even in old cultures it is only partial.

Remarks. In the description of *Micrococcus* of Freire, Dr. Sternberg states that milk is not coagulated, but this was not the case with the authentic culture possessed by the college. It reacted in all ways as given in the synopsis and as indicated above.

18. *Merismopedia acidi lactici liquefaciens* (Kreuger).

Occurrence. (1) "*Micrococcus ureae*" from Král's laboratory. (2) "*Sarcina erythromyxa*" from Král's laboratory.

Morphology. Micrococci $.9-1.1\mu$ in diameter, singly, in twos or in fours.

Biology. Gelatin is quickly liquefied but in (2) proceeded slowly and was not complete. Milk is coagulated, forming a solid curd, but in (2) only so on boiling. Nitrate very slightly reduced; rosolic acid unchanged. Growth on solid media opaque white; in broth, a white precipitate with a slight growth at the surface or on the side of the tube. Grows well at $37\frac{1}{2}^{\circ}\text{C}$.

Remarks. I see no essential differences between the descriptions of *Micrococcus acidilactici liquefaciens* Kreuger, *Micrococcus ureae liquefaciens* Flügge, or *Sarcina alba* Eisenberg, to either of which the present species might be referred. It does not belong to either of the species under the names of which it was sent to me.

19. *Micrococcus flavus liquefaciens* (Flügge).

Occurrence. In the air of an apartment house on West 69th St.

Morphology. Large spherical Micrococci, $1-1.25\mu$ in diameter, singly, in pairs or irregular groups.

Biology. Liquefaction of the gelatin takes place very slowly. When first obtained, it ensued in ten days, but proceeded very slowly. After the culture had been kept a year, liquefaction was not obtained until the two cultures which had been made had been growing twenty-eight and fifty days respectively. A very slight reduction of nitrate was obtained in twenty-eight days, none before that time. Growth on solid media, white at first, later becoming pale yellow. Growth on glycerine agar and at $37\frac{1}{2}^{\circ}\text{C}$. is white, but in the latter case the yellow color is regained on being transferred to the room temperature.

Remarks. This differs a little from Flügge's species with which I have identified it, but apparently only in liquefying gelatin more slowly and being less chromogenic. No growth was obtained on potato.

20. *Micrococcus flavus desidens* (Flügge).

Occurrence. In the air of an apartment house on West 69th St.

Morphology. Small Micrococci, $.6-.7\mu$ in diameter, in irregular groups.

Biology. As indicated in the synopsis. The liquefaction of gelatin is very slow and only appears in old cultures (first seen in fifty-six days). A partial reduction of nitrate is obtained in twenty-eight days. On solid media the growth is lemon yellow, rather thin, poor on glycerine agar; on potato slight growth, yellowish, but the potato assumed a pink tint.

Remarks. It differs from the species with which I have identified it in liquefying gelatin much more slowly.

21. *Merismopedia fragilis* n. sp.

Occurrence. In the air on 59th St.

Morphology. Micrococci $1-1.2\mu$ in diameter, in twos, fours and irregular groups but scarcely forming well defined packets.

Biology. Gelatin liquefied rather slowly, but beginning in six days; characters all negative except the reddening of lactose-litmus, and this is not of a very pronounced character. Growth on agar poor, thin, translucent, pink-

ish, consisting of a number of isolated colonies, each spreading with a veil. In gelatin the growth along the punctures is white, the surface growth sinks slowly in the liquefaction as a pinkish sediment. On glycerine agar the growth is abundant, thick, smooth and of a reddish brown color. Scarcely any growth on potato; no growth at the body temperature.

22. *Merismopedia citreus conglomeratus* (Bumm).

Occurrence. In the air of the college hallway.

Morphology. Micrococci .9 μ in diameter, singly, in twos, rarely in elliptical pair with a cross furrow.

Biology. Gelatin liquefied quickly at first (six days), but in latter cultures not till forty-five days; nitrate reduced, partially at first, but in twenty-eight days completely. Gelatin plate colonies are at first white, opaque, very strongly cumular and lobed; they are unusually coherent and will flow around in the liquefied medium with unaltered shape. On agar the growth is white at first, later distinctly pale yellow, granular and lumpy, somewhat brittle and crusty to the needle. Rosolic acid is rendered a little brownish but not distinctly faded.

Remarks. This does not tally exactly with Bumm's description of *Diplococcus citreus conglomeratus*, but I find no positive points of difference.

23. *Merismopedia mollis* n. sp.

Occurrence. As a contamination from the air of the laboratory.

Morphology. Micrococci, sometimes a little elongate, about 1 μ in diameter, singly, in pairs and a few fours, in irregular chains of four to six and in masses.

Biology. Gelatin quickly liquefied; milk coagulated, the precipitated case in forming a curd which is gradually dissolved. Nitrate partly reduced. Lactose-litmus and rosolic acid unchanged. Growth on agar abundant, shining orange color.

24. *Sarcina flava* (De Bary).

Occurrence. (1) "*Sarcina ventriculi*" from the college collection. (2) Four different times in the air of the college hallway. (3) In the air in West 59th St. (4) Twice as a contamination from the air of the laboratory on gelatin plates. (5) In a fresh leaf of the pitcher plant, *Sarracenia purpurea*, at Plattsburgh, N. Y., in June.

Morphology. Micrococci about 1 μ in diameter associated in cubical packets of eight and larger bundles, rarely breaking up into fours.

Biology. Gelatin usually quickly liquefied, more rarely slowly liquefied; milk coagulated, the precipitated casein forming a curd which is gradually dissolved. Nitrate not reduced or but a trace. Lactose-litmus made blue; rosolic acid not changed. Growth on agar abundant, thick, not very shining, yellow; on glycerine agar very abundant, shining bright yellow. At 37½° C the growth is white, but largely regains its yellow color on being transferred to the room temperature.

Remarks. I find no characters to separate *Sarcina lutea* Schröter from this species. It appears to liquify gelatin more slowly ; but the cultures before me vary greatly in this respect. One produced only a dry cup-shaped hollow in twenty-eight days, from which the fluid at first formed had evaporated. The more usual form rendered the upper one-third or two-thirds of the gelatin fluid in twenty-eight days.

25. *Micrococcus dissimilis* n. sp.

Occurrence. "Micrococcus of trachoma, Sattler" from Král's laboratory.

Morphology. Micrococci 1μ in diameter, associated in irregular masses.

Biology. Gelatin quickly liquefied ; milk coagulated, forming a flaky curd with milky whey ; nitrate reduced, but not thoroughly before twenty-eight days ; lactose-litmus and rosolic acid unchanged ; opaque white on agar, not viscid ; surface colonies round, opaque, like deep ones but surrounded by an obscure granular veil ; no surface growth on broth. No growth on potato.

Remarks. This does not agree with the description of the *Micrococcus* of trachoma.

26. *Micrococcus mobilis* (Maurea).

Occurrence (1) Twice in the air of the college hallway. (2) Twice in the air on 59th St.

Morphology. Micrococci $1-1.5\mu$ in diameter, singly, rarely in pairs. Each cell possesses a single long flagellum which may be demonstrated by Löffler's method of staining ; actively motile.

Biology. Gelatin quickly liquefied, or, in one instance, slowly and imperfectly ; rosolic acid not changed. On agar the growth is translucent, obscure, where collected in a thicker layer reddish orange. On glycerine agar and at $37\frac{1}{2}^{\circ}$ scarcely any growth occurs ; growth on potato very slight, pale orange. In absence of free access of air, the growth is whitish.

Remarks. I take this to be the *Sarcina mobilis* of Maurea.

27. *Micrococcus agilis* (Ali-Cohen).

Occurrence. From the college collection.

Morphology and biology as above (No. 26), but the growth is pink instead of orange color and is thicker and more opaque on agar.

28. *Sarcina aurantiaca* (Koch).

Occurrence. (1) From the college collection. (2) In the air of the college hallway.

Morphology. Micrococci $.8-1.2\mu$ in diameter, in twos, fours, and masses, occasionally forming true packets.

Biology. Gelatin quickly liquefied (1) or slowly and imperfectly (2). Nitrate not reduced, or a slight trace of reduction. On agar the growth is dark orange colored, not very shining. Rosolic acid not changed. In broth no surface growth, the sediment yellowish or white.

29. **Merismopedia rosea** (Bumm).

Occurrence. (1) "*Sarcina rosea*" from the college collection. (2) "*Micrococcus tetragenus ruber*" from Krål's laboratory. (3) Twice in the air of the college hallway. (4) In the air of an apartment house on West 69th St. (5) A contamination from the air of the laboratory.

Morphology. Micrococci .7-1 μ in diameter, in twos, fours and irregular groups.

Biology. Gelatin is very slowly liquefied, often not at all for thirty or forty days. Some cultures liquefy more readily after having been in cultivation for some time. Nitrate is partly reduced, sometimes quite well, never completely. Rosolic acid not changed. Growth on solid media rose pink, the color being somewhat absorbed by the medium in old cultures. No surface growth on broth.

Remarks. I have identified this as the *Diplococcus roseus* of Bumm. *Micrococcus roseus* Eisenberg, and *Micrococcus tetragenus ruber* Schneider seem to refer also to this species.

30. **Micrococcus tetragenus vividus** n. sp.

Occurrence. (1) Four times in the air of the college hallway. (2) In the air of an apartment house on West 69th St. (3) From the air of one of the lecture rooms (Dr. Cheesman).

Morphology. Small Micrococci .5-.9 μ in diameter, singly, in twos, threes or irregular groups, rarely in fours.

Biology. Gelatin is liquefied with moderate rapidity, or rather slowly; nitrate not or very slightly reduced. Growth on solid media vivid lemon yellow; in broth a heavy yellow sediment without surface growth.

Remarks. Dr. Sternberg has included both this form and No. 32 in his description of *Micrococcus tetragenus versatilis*, but I have preferred to give them distinctive names, following the general system adopted here.

31. **Micrococcus tetragenus versatilis** (Sternberg).

Occurrence. (1) "*Micrococcus tetragenus versatilis*" from the college collection. (2) "*Micrococcus tetragenus flavus*" from Krål's laboratory. (3) Five times in the air of the college hallway. (4) In the air on 59th St.

Morphology and *biology* as in No. 30, but the growth is paler yellow of a more normal tint, not unusually bright.

32. **Micrococcus tetragenus pallidus** n. sp.

Occurrence. In the air of one of the college lecture rooms (Dr. Cheesman).

Morphology. Micrococci .8-1 μ in diameter, in twos, more commonly in fours, occasionally in irregular packets.

Biology. As in Nos. 30 and 31, but the growth is pale creamery yellow, approaching white.

33. *Merismopedia mesentericus corrugatus* n. sp.*

Occurrence. "Staphylococcus pyogenes citreus" from the college collection.

Morphology. Micrococci .9 μ in diameter, associated in great adherent masses more or less packeted, breaking apart into twos or single elements.

Biology. Gelatin is quickly liquefied. On agar, the growth spreads rapidly, and becomes covered with a few large wrinkles or folds, traversing the surface in various directions; the color is yellow. On broth, a thick and wrinkled yellow surface skin is formed with a slight yellow sediment in the bottom.

NON-LIQUEFYING BACILLI.

(Synopsis of species.)

Lactose-litmus reddened.

Nitrate not reduced.

Milk coagulated 34

Milk not coagulated.

Actively motile, a green fluorescence..... 35

Not motile or spasmodically only.

Small bacilli..... 36

Very large vacuolated 37

Nitrate reduced, at least partially.

Milk coagulated.

Lactose-litmus permanently reddened, growth white..... 38

Lactose-litmus reddened, later blue, growth sometimes faintly
ocherous..... 39

Milk not coagulated.

Growth yellow, nitrate completely reduced..... 40

A green fluorescence; nitrate partly reduced..... 41

Pale yellow, nitrate slightly reduced..... 42

Lactose-litmus made blue.

Nitrate reduced, at least fairly well; milk not coagulated.

Growth dark yellow..... 43

Growth pale yellow..... 44

Growth white.

Grows well at room temperature.

Rosolic acid faded..... 45

Rosolic acid not faded.

Not motile..... 46

Actively motile.

Growth thin, transparent..... 47

Growth moderately opaque..... 48

Scarcely grows at room temperature..... 49

* Compare with this No. 107, *Bacillus erythrogenes rugatus*. The biological characters of the two are practically alike. No. 33 was determined as a *Micrococcus*, and No. 107 as a *Bacillus*; but it is one of the shortest bacilli known, so that the differences between these forms are really slight. No. 33 was not observed to form any pink color; but this is not an invariable character of the *lactis erythrogenes* group.

Nitrate not reduced or not more than very slightly.

Chromogenic.

Motile ; a green fluorescence.

Rosolic acid decolorized..... 50

Rosolic acid not changed.

Growth white. 51

Growth yellow 52

Not motile.

A crusty skin on broth more or less complete.

Orange red..... 53

Pale whitish orange..... 54

Slightly crusty, brick red 55

No crusty skin.

Salmon pink, pale along puncture..... 56

Reddish pink, white along puncture..... 57

Pink, white along puncture..... 58

Bright orange 59

Yellow 60

Not chromogenic.

Rosolic acid decolorized.

Culture viscid 61

Culture soft 62

Rosolic acid not changed.

Growth very feathery, especially in gelatin..... 63

Growth not feathery, normal.

Motile ; growth white, rather opaque.

Rather large bacilli (about $.8 \times 1.5 \mu$)..... 64

Smaller bacilli (about $.5 \times 1 \mu$)..... 65

Not motile.

White, rather opaque 66

Misty, concentric, not opaque..... 67

Very thin, transparent..... 68

34. *Bacillus crassus sputigenus*. (Kreibohm).

Occurrence. From the college collection.

Morphology. Short, or moderately long rounded bacilli, $.7 \times 1-2 \mu$, singly, or more commonly in short chains of two to three elements ; not motile.

Biology. As indicated in the synopsis. Milk was not coagulated till twenty-eight days and then only on boiling.

35. *Bacillus virescens* (Frick).

Occurrence. " *Bacillus* of green diarrhoea " from the college collection.

Morphology. Small rounded bacilli, $.7 \times 1-2 \mu$, singly or in moderately long straight chains ; actively motile.

Biology. A partial surface skin is formed on liquid media. In the fermentation tube, abundant growth occurs in the open arm, but it also extends well up into the closed arm. Growth on agar white, rather translucent,

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a green fluorescence in the medium. Lactose-litmus is reddened rather slowly, but ultimately completely.

Remarks. From the description, this culture can be referred to either *B. fluorescens tenuis* Zimmerman, *B. virescens* Frick, or *B. dentalis viridans* Miller. The "Bacillus of Lesage" (green diarrhoea) is said to produce slow liquefaction. It differs from No. 41 only in that nitrate is not reduced.

36. **Bacillus sarracenicolus** n. sp.

Occurrence. In a fresh leaf of the pitcher plant (*Sarracenia purpurea*) at Plattsburgh, N. Y., in June.

Morphology. Small bacilli, surrounded by clear spaces in the stained preparation, not motile; in pairs, short chains or singly; size about $.5 \times 1 \mu$.

Biology. Growth on agar, white, faintly tinged with brownish, spreading; when old, almost ocherous; on lactose-litmus gelatin the growth spreads out into long lobes, the litmus is reddened, but the color begins to disappear in forty days, and the medium is purple or bluish in ninety days.

37. **Bacillus vacuolatus** n. sp.

Occurrence. Two cultures from a trap of the carnivorous water plant *Utricularia vulgaris* from Dead Creek, Plattsburgh, N. Y., in September.

Morphology. Large bacilli varying greatly in length, about 1μ wide and 1.5μ long, the ends rounded; the contents are curiously vacuolated both in fresh and old cultures, so that the bacilli stain irregularly, but usually well at the ends; singly or in short chains; usually not motile, but occasionally spasmodically so. Looks like the figure of *Bacillus buccalis marinus*, Miller.

Biology. On agar shining, soft translucent white. Lactose-litmus gelatin is reddened, but in about sixty days this color begins to be replaced by blue which gradually increases, though the red was still visible at the apex of the slant in ninety days.

38. **Bacillus ureæ** (Jaksch).

Occurrence. (1) "Bacillus ureæ" from Krål's laboratory. (2) "Bacillus pyogenes foetidus" from Krål's laboratory.

Morphology. Short rounded bacilli $.7-1 \times 1-2 \mu$, not motile, singly or in short chains.

Biology. Nitrate partially reduced, the test giving a moderate color; milk coagulated in (2) only on boiling. Growth on agar, broad shining sordid white, not very opaque; no surface growth on broth; rosolic acid unchanged. A considerable amount of indol is formed in peptone-salt broth.

Remarks. Appears not to be contradicted by the description of *Bacterium ureæ* of Jaksch. Comes near *Bacillus ubiquitous* Jordan, but possesses much less power of reducing nitrate.

39. **Bacillus subochraceus** n. sp.

Occurrence. In the air of the college hallway.

Morphology. Rather small bacilli, $.7 \times 1.5 \mu$, not motile or spasmodically so, singly or in short chains.

Biology. Milk is not coagulated at first, but in twenty-six days a coagulum was obtained on boiling; only very slight nitrate reduction. Litmus is reddened at first, but in thirty-five days the red begins to disappear and is replaced by purple, still later by blue (ninety days). Growth on solid media is translucent with more or less of an ochereous tint, sometimes almost light orange (lactose-litmus gelatin). There is a slight surface growth on broth; rosolic acid is deepened in color. Surface colonies on gelatin are clear, rather irregular, slightly veined; the deep ones are round, dusky yellowish.

40. **Bacillus domesticus** n. sp.

Occurrence. In the air of an apartment house on West 69th street.

Morphology. Rather small rounded bacilli, $.5 \times 1 \mu$, mostly in pairs, not motile.

Biology. Nitrate is reduced rather slowly, but completely in seventeen days. A slight pink tint may be seen in the surface of milk. Litmus is made red but in fifty days begins to be replaced by blue. Growth on solid media at first white, but later shining light yellow, slowly spreading. Grows well on potato and glycerine agar. The surface colonies on gelatin are larger than the deep ones, thin, granular yellowish centrally with even edge.

Remarks. Seems near *B. striatus flavus* von Besser, but the cells are not curved and I did not notice any striations.

Also near No. 42, but the nitrate is much more strongly reduced.

41. **Bacillus fluorescens tenuis** (Zimmermann).

Occurrence. "Micrococcus versicolor" from the college collection.

Morphology. Rather short bacilli, mostly in short chains, $.6 \times 1-2 \mu$ actively motile.

Biology. Nitrate quite strongly reduced but not completely. Growth on agar translucent white with a green fluorescence in the medium; lactose-litmus permanently reddened.

Remarks. See remarks under No. 35.

42. **Bacillus amabilis** n. sp.

Occurrence. In the air of the college hallway.

Morphology. Very short bacilli, often nearly spherical, $.7 \times .8-1 \mu$ singly or in irregular chains or masses; not motile.

Biology. Nitrate slightly reduced. On agar a narrow translucent white streak with pale yellow tint; surface colonies rather large, translucent, yellowish, on potato a very thin growth, bright yellow with ill defined edges. Produces no indol; rosolic acid not changed.

Remarks. The cultures died during the summer vacation.

43. **Bacillus flavocoriaceus** (Eisenberg).

Occurrence. In the air of an apartment house on West 69th street.

Morphology. Rounded bacilli, singly or in masses $.6 \times 1-1.5 \mu$.

Biology. Nitrate quite well reduced but not completely. Growth on solid media rather dark yellow, shining, the edges inclined to be translucent. Scarcely any growth was obtained on potato. Rosolic acid a little deepened in color.

44. **Bacillus javaniensis** n. sp.

Occurrence. "Photobacterium javaniensis" from the college collection.

Morphology. Short elliptical bacilli, almost like cocci, about $1-1.2 \mu$ in diameter, in masses, short chains, twos or fours, not motile.

Biology. Nitrate is strongly reduced but not completely; rosolic acid unchanged. Growth on agar thick, white, with a distinct yellow tinge; grows slowly.

Remarks. I have met with no description of this species and apply the name with which it was labelled.

45. **Bacillus decolorans minor** n. sp.

Occurrence. In the air of an apartment house on West 69th street.

Morphology. Rounded bacilli $.6 \times .6-1.1 \mu$, frequently in pairs, not motile.

Biology. Nitrate slowly reduced but completely in twenty-eight days; rosolic acid decolorized; indol formed in peptone-salt broth. In gelatin cultures, surface growth waxy white, not shining, irregular and finely lumpy, slight growth along puncture. Surface colonies large, spreading, translucent white with irregular edges; deep ones dusky yellowish, not characteristic. On agar translucent whitish, rather thin, shining. On potato very slight brownish translucent growth, not abundant.

46. **Bacillus secundus Fullesi** n. sp.

Occurrence. In the air of the college yard near 59th street.

Morphology. Large bacilli, short, rounded, singly, often constricted, $.7-1 \times 1-2 \mu$; not motile.

Biology. Nitrate quite well reduced but not completely; rosolic acid not faded. Growth on agar white, rather thin, lactose-litmus made blue.

Remarks. Seems to be not contradicted by the description of *Bacillus* No. II. of Fulles.

47. **Bacillus aquatilis sulcatus quartus** (Weichselbaum).

Occurrence. In a trap of *Utricularia vulgaris*, Plattsburgh, N. Y., in September.

Morphology. Rounded bacilli, $.6 \times 1.5 \mu$, in pairs or short chains; in older cultures growing out into long straight chains; the free cells actively motile.

Biology. Nitrate partly reduced; rosolic acid unchanged.

Remarks. I have applied the name *B. aquatilis sulcatus*, No. IV., of Weichselbaum, to this form, as it seems not to positively contradict the description.

48. **Bacillus primus Fullesi** n. sp.

Occurrence. In a leaf of *Sarracenia purpurea* at Plattsburgh, N. Y.

Morphology. Small rounded bacilli, singly and in pairs, do not form long chains; $.5-.6 \times .8-1 \mu$, actively motile, but becoming much less so in old cultures (twenty-five days).

Biology. Nitrate reduced, at length completely, rosolic acid not changed; the cultures in milk emit a disagreeable odor.

Remarks. The *Bacillus* No. I., of Fulles, seems to correspond.

49. **Bacillus diphtheriæ** (Klebs).

Occurrence. From the college collection.

Morphology. Short bacilli, nearly spherical, irregular; $.8 \times 1 \mu$ (grown at room temperature).

Biology. Grows very poorly at room temperature so that the characters are not all reliable. Nitrate well reduced in twenty-eight days but not completely.

50. **Bacillus erythrosporous** (Eidam).

Occurrence. From the college collection.

Morphology. Rounded bacilli, $.6 \times 1-1.5 \mu$, singly or in short chains; motile, form spores.

Biology. A slight trace of reduction of nitrate; rosolic acid decolorized; lactose-litmus made blue at first, but in sixty days the color becomes purple, and in one hundred days bright red. Growth on agar translucent white, a green fluorescence in the medium.

51. **Bacillus fluorescens putidus** (Flügge).

Occurrence. (1) From the college collection. (2) From Král's laboratory at Prague. (3) In the exudation from the anus of some sick lepidopterous larvæ (*Clisiocampa fragilis*) bred in confinement.

Morphology. Rounded bacilli, $.6-.8 \times 1-2 \mu$, singly or in short chains, actively motile.

Biology. Nitrate not reduced, rosolic acid not changed; lactose-litmus blue at first but begins to redden in sixty to ninety days. The reddening became most pronounced in culture (3). Agar growth translucent white with a green fluorescence in the medium.

52. **Bacillus fluorescens aureus** (Zimmermann).

Occurrence. From the college collection.

Morphology. Large, rather slender bacilli. $.7-1 \times 1-5 \mu$, singly or in chains; spsmodically motile, the longer chains bending at the joints.

Biology. As above, but the growth is yellow. The description says ochreous or golden yellow, but the culture before me has become a very pale yellow. No sign of reddening of lactose-litmus in forty-five days.

53. **Bacillus fuscus** (Zimmermann).

Occurrence. (1) "*Bacillus chrysogloia*" from Krål's laboratory. (2) In the air of the college hallway. (3) A contamination from the air of the college laboratory.

Morphology. Rounded bacilli, $.5-.7 \times 1-2\mu$, singly or in chains of various lengths; not motile.

Biology. Nitrate not reduced or but slightly. Growth characteristically wrinkled with lobed edges, of crusty brittle texture forming a surface skin on liquid media; color light orange. Rosolic acid not changed. Aerobic.

Remarks. Does not correspond to the description of *B. chrysogloia* Zopf.

54. **Bacillus fuscus pallidior** n. sp.

Occurrence. "*Bacillus latericeus*" from Krål's laboratory.

Morphology. Rounded bacilli $.5-.7 \times 1-1.3\mu$, singly and in chains.

Biology. As in No. 53, but the growth is a very pale whitish orange, almost pinkish.

Remarks. Does not correspond to *B. latericeus* Eisenberg.

55. **Bacillus ferrugineus** n. sp.

Occurrence. (1) A contamination from the air of the college laboratory. (2) From a fresh leaf of *Sarracenia purpurea* at Plattsburgh, N. Y.

Morphology. Little rounded bacilli $.6 \times 1\mu$, in pairs or chains of various lengths; not motile.

Biology. Much as in Nos. 53 and 54 but the growth is red, brick red instead of orange; it is crusty or granular, scarcely wrinkly as the preceding are; grows slowly. Nitrate not reduced, milk not changed, but an abundant brick-red growth forms on the surface. In broth a complete surface skin is not formed. The gelatin colonies under a low power appear like a round tuft of cotton but small and regular.

56. **Bacillus salmoneus** n. sp.

Occurrence. In the air of the college hallway.

Morphology. Small rounded bacilli, $.5 \times .7\mu$, singly or in short chains, not motile.

Biology. A slight reduction of nitrate; growth on agar narrow, shining, salmon pink, quite opaque where thick. No surface growth on broth. Deep gelatin colonies rounded, regular, smooth, uncharacteristic.

57. **Bacillus finitimus ruber** n. sp.

Occurrence. (1) Four times in the air of the college hallway. (2) In the air on West 59th Street. (3) "*Micrococcus cinnabareus*" from the college collection.

Morphology. Short rounded bacilli $.5 \times .6-1\mu$, singly or in chains of three or four, not motile.

Biology. Nitrate not reduced but in one instance considerably reduced. Resembles Nos. 56 and 58 but the color of the growth is reddish pink or rather bright red.

58. **Bacillus rhodochrous** (Overbeck).

Occurrence. (1) "Micrococcus rhodochrous" from Krål's laboratory. (2)

In the air of the college hallway.

Morphology and biology as above, but the growth is a fine pink with the free access of air.

59. **Bacillus brunneoflavus** n. sp.

Occurrence. "Micrococcus brunneus" from Krål's laboratory.

Morphology and Biology. As in Nos. 57 and 58 but the growth is bright orange. There is a slight reduction of nitrate; rosolic acid not changed.

Remarks. Does not correspond with *Bacillus brunneus* Adametz.

60. **Bacillus flavocoriaceus** (Eisenberg).

Occurrence. In the air of the college hallway.

Morphology. Very short bacilli, regular and uniform, $.5 \times .7 \mu$, singly or in chains of two or three; not motile.

Biology. Characters all negative. Grows well on glycerine agar, but not on potato or at $37\frac{1}{2}^{\circ}$ C.

Remarks. The gelatin colonies are not coarsely granular, but smooth. In other respects, the description of Eisenberg is not contradictory.

61. **Bacillus zürnianum** (List).

Occurrence. In the air of the college hallway.

Morphology. Small rounded bacilli, $.5 \times .6-1 \mu$, singly or in short chains, not motile.

Biology. A very slight reduction of nitrate. Cultures on solid media translucent white, very viscous, drawing out into long threads. Does not produce indol in peptone-salt solution.

Remarks. These bacilli are not as long as *B. zürnianum* List but seems to correspond otherwise.

62. **Bacillus decolorans major** n. sp.

Occurrence. (1) In the air of an apartment house on West 69th Street. (2)

In the air of the college hallway.

Morphology. Very short rounded bacilli $.7-9 \times 1-2 \mu$, singly or in chains of various lengths, not motile.

Biology. As in No. 61, except that the growth is not in the least viscous, and indol is formed in the peptone-salt broth. The decolorization of rosolic acid does not take place at once but is complete in from sixteen to twenty-five days.

Remarks. Differs from No. 45 in that the cells are larger and that nitrate is not reduced or but partially so.

63. **Bacillus zopfii** (Kurth).

Occurrence. (1) "Bacterium zopfii" from the college collection. (2) "Proteus zenkeri" from the college collection. (3) "Proteus mirabilis"

from the college collection. (4) "*Bacillus ramosus non-liquefaciens*" from Král's laboratory. (5) "*Bacillus figurans*" from the college collection.

Morphology. Large straight rod-shaped bacilli $.8 \times 1-4\mu$, but soon breaking up into shorter cells in older cultures; singly or in chains; motile. In a twenty-one day culture the bacilli were very short, of the shape of spores but more spherical.

Biology. Nitrate not reduced or but slightly. The growth on gelatin, both colonies and streak is very characteristic, sending off fine ramifying branches far out into the medium. On agar the growth is thin, white, more or less feathery on the edges. Indol is formed. Lactose-litmus quickly made blue.

Remarks. *Proteus zenkeri* is probably the same species as *B. zopfii*. The other cultures before me seem to be wrongly named.

64. *Bacillus lactis cyanogenus* (Hueppe).

Occurrence. (1) "*Bacillus* of blue milk" from the college collection. (2) "*Bacillus cuniculicida*" from Král's laboratory.

Morphology. Rounded, thick bacilli, $.7-1 \times 1-1.5\mu$, singly or in pairs, actively motile.

Biology. Nitrate not reduced or but slightly; aerobic, as shown by the growth in the fermentation tube. A slight surface growth on broth. Agar growth translucent white.

Remarks. The characteristic blackish color was produced in none of my media, although I made a culture in milk with an acid forming bacillus as recommended. The cells were twice as thick as described by Hueppe, but corresponded with the measurement of Jordan. I was unable to differentiate the culture sent to me as "*Bacillus cuniculicida*" from this species, as neither produced the black color, and they corresponded very closely otherwise. This culture does not correspond with the description of *B. cuniculicida* (*B. septicaemiæ hæmorrhagiæ* according to Sternberg), as this is said to be not motile.

65. *Bacillus typhi abdominalis* (Eberth).

Occurrence. (1) "*Bacillus typhosus*" from the college collection. (2) In the air of the college hallway. (3) From a fresh leaf of *Sarracenia purpurea* at Plattsburgh, N. Y.

Morphology. Slender rounded bacilli $.5-.7 \times 1-2\mu$, singly or in chains; motile. In No. 3 the cells measure about $.5 \times .7\mu$ only.

Biology. Nitrate not reduced or partially so in fifty days. Growth on solid media translucent white.

Remarks. I do not wish to be understood to imply that the bacilli found by me in the air and the pitcher plant would produce typhoid fever, or even that they are pathogenic. The presumption is that they are not, and I have only included them here because they did not differentiate themselves on the media used. In this group, the characters are all negative, and we may have to do with closely allied species. I have tried to apply

the characters uniformly in all cases, and I think we may learn as much from cases where separations apparently fail to be made as where they come out unexpectedly.

66. **Bacillus candicans** (Frankland).

Occurrence. (1) Twice in the air of West 59th Street. (2) In the air of the college (Dr. Cheesman). (3) A contamination, from the air?

Morphology. Short rounded bacilli, $.5-.8 \times .8-1.3\mu$, singly or in twos or threes; not motile.

Remarks. Resembles No. 62, but rosolic is not decolorized.

67. **Bacillus Martinezii** (Sternberg).

Occurrence. A contamination from the air of the college laboratory.

Morphology. Rounded bacilli, $.5 \times 1-1.5\mu$, singly or in short straight chains; not motile.

Biology. Nitrate slightly reduced in twenty-eight days; rosolic acid deepened in color; growth on agar translucent white. Gelatin colonies are large, irregular streaked, almost reticulated, with irregular edge; the mass has a veriform appearance.

Remarks. As Sternberg's description does not seem to contradict this culture in any essential feature, I have applied his name to it, though I have no other reason for supposing them to be the same.

68. **Bacillus inutilis** n. sp.

Occurrence. Three colonies on some plates exposed to the air in West 59th Street, and grown at $37\frac{1}{2}^{\circ}$ C.

Morphology. Short, rounded bacilli, rather large, $1 \times 1-2\mu$, singly and in pairs.

Biology. Characters all negative, growth forms uncharacteristic, on solid media very thin, translucent; grows both at room temperature and at $37\frac{1}{2}^{\circ}$ C.

LIQUEFYING BACILLI.

(Synopsis of species.)

Nitrate not reduced or but very slightly.

Milk not coagulated.

Lactose-litmus not reddened.

Not chromogenic.

Large bacilli, $.5\mu$ wide or more; form spores.

Growth skinny; a surface skin on broth (see 88 and 89).

Growth not skinny or crusty.

Growth viscous; not motile. 69

Growth not viscous; motile.

Shining translucent white on agar.

No growth on potato. 70

Growth on potato abundant, but nearly invisible. . 71

Very thin and transparent on agar

No growth on potato 72

An abundant growth on potato like brown varnish. 73

Very small bacilli ; not motile.....	74
Chromogenic.	
A greenish fluorescence.....	75
Color orange-yellow, growth skinny.....	76
Color orange or brownish, growth soft.....	77
Color yellow (see 104 to 107).	
Lactose-litmus reddened ; chromogenic ; not motile.	
Growth thin, translucent, reddish where thick.....	78
Growth yellow, rather opaque.....	79
Milk coagulated ; always so on boiling.	
Lactose-litmus reddened.	
A green fluorescence ; also a black pigment in presence of sugar	80
Not fluorescent ; more or less chromogenic.	
Motile.....	81
Not motile.	
Growth ochreous, faintly chromogenic.....	82
Growth translucent salmon-pink.....	83
Growth brownish red or dark orange-red.....	84
Lactose-litmus reddened.	
Chromogenic ; yellow.....	85
Not chromogenic.	
No complete surface skin on broth.	
Growth white, marked with more opaque white.....	86
Growth very thin and translucent.....	87
A wrinkly surface skin on broth.	
Spore-bearing cells wider than vegetative ones.....	88
Cells alike, the spore-bearing ones distended by the spores.	89
Nitrate reduced, at least partially in twenty-eight days.	
Milk coagulated, especially so on boiling.	
Growth white, waxy, dotted or feathery.	
Growth rather thin, granular, dotted or punctate.....	90
Growth more opaque like ground glass, more or less feathery.	
Growth subcrystalline, finely angularly marked.....	91
Growth scarcely more than finely granular, uniform.	
Not, or but slightly wrinkly.....	92
With transverse, convex folds or wrinkles.....	93
Growth rather opaque, feathery, with concave creases.....	94
Growth very thin, spreading.....	95
Chromogenic, sometimes only faintly so.	
Color ochreous.	
Long, slender bacilli.....	96
Very small, short bacilli (see 77).	
Color pinkish red, rarely white.	
Growth viscous.....	97
Growth not viscous.....	98

Color blackish violet.....	99
Faintly chromogenic, creamy when old.	
Growth moderately opaque ; no fluorescence,	
Growth smooth, shining	100
Growth coarsely granular, the lobed parts nearly smooth...	101
Granular punctate with impressed wrinkles ; no lobes.....	102
Growth rather thin ; a green fluorescence	103
Milk not coagulated ; if apparently so, no firm coagulum on boiling.	
Yellow ; milk often distinctly coagulated in the cold, but not on boiling.	
Lactose-litmus made blue.	
Growth, soft, smooth, shining.	
Gelatin quickly liquefied	104
Gelatin slowly liquefied.....	105
Growth lumpy, granular	106
Growth heavily erased (convex).....	107
Lactose-litmus reddened ; growth viscous	108
A green fluorescence in the medium	109
Bright orange ; growth forming a crusty skin.....	110
Faintly chromogenic or not.	
Rosolic acid unchanged or darkened	111
Rosolic acid decolorized... ..	112

69. *Bacillus vermiculosus* (Zimmermann.)

Occurrence. From Krål's laboratory.

Morphology. Square ended bacilli, $1 \times 2-4 \mu$, singly or in chains, not motile.

Biology. Gelatin slowly liquefied, not complete in twenty-eight days ; usually begun in fourteen days. The growth on agar is viscous, coming off in long strings on the needle. Rosolic acid not changed. On potato the growth is broad and thick of a sordid flesh color.

70. *Bacillus alpha* n. sp.

Occurrence. In the air on West 59th Street.

Morphology. Large bacilli, $.8 \times 1-2 \mu$, singly or in long close straight chains ; motile.

Biology. Gelatin not liquefied at first, but well liquefied in one-hundred days. Some colonies on a gelatin plate liquefied in eleven days. Rosolic acid not changed ; produces some indol.

71. *Bacillus beta* n. sp.

Occurrence. In the air of the college hallway.

Morphology. Short, large bacilli with rounded ends, $.6 \times 1.5-2 \mu$, actively motile, forms spores.

Biology. Gelatin liquefied rather slowly, the surface growth on this medium very feathery and thin without definite boundary. Rosolic acid not changed ; forms some indol.

72. **Bacillus circulans** (Jordan).

Occurrence. In the air of the college hallway.

Morphology. Large bacilli with rounded ends, $.6 \times 1-2\mu$, mostly singly; motile.

Biology. Gelatin rather slowly liquefied; milk not coagulated; nitrate very slightly reduced in twenty-eight days; rosolic acid not decolorized; forms a little indol. Growth on agar thin and translucent.

73. **Bacillus Scheurleni** (Sternberg).

Occurrence. In the air on West 59th Street.

Morphology. Large rounded bacilli, $.7-1 \times 1-2.5\mu$, singly and in short chains motile; forms spores.

Biology. Gelatin quickly liquefied; rosolic acid not changed, forms no indol, nitrate very slightly reduced in twenty-eight days.

Remarks. Apparently corresponds to the "Bacillus of Scheurlen" described in Sternberg's manual.

74. **Bacillus incanus** (Pohl).

Occurrence. In a leaf of *Sarracenia purpurea* at Plattsburgh, N. Y.

Morphology. Small rounded bacilli, $.4-.5 \times .6-1\mu$, usually in short chains, not motile, does not form spores (115 days on agar).

Biology. Gelatin liquefied rather slowly, nitrate slightly reduced, rosolic acid not changed; growth on agar translucent white, not broad, irregularly streaked.

Remarks. I have identified this with *B. incanus*, though with some hesitation, as that species is said to be slightly motile, and the growth on agar is described as granular.

75. **Bacillus fluorescens nivalis** (Schmolek).

Occurrence. In the exudate from a sick lepidopterous larva (*Scoliopteryx libatrix*) at Keene Valley, N. Y.

Morphology. Rather slender bacilli, $.5-.7 \times 1-3\mu$, mostly singly, actively motile.

Biology. Gelatin quickly liquefied; rosolic acid nearly decolorized in sixteen days; nitrate not reduced; growth on agar thin and translucent, a fine greenish fluorescence in the medium.

Remarks. Differs from No. 109 in not reducing nitrate. I have identified this as above rather than describe it as new, though there is nothing in the description to differentiate it from No. 109.

76. **Bacillus gamma** n. sp.

Occurrence. In the air of the college yard, near West 59th Street.

Morphology. Bacilli associated in thick adherent masses, $.5-.7 \times 1-1.5\mu$, motile, in old cultures many small spherical forms are seen.

Biology. Milk not coagulated, but the casein ultimately dissolved; lactose-litmus is made blue, though sometimes this is obscured by the decoloring effect; nitrate not reduced, rosolic acid not changed. The species is

aerobic and forms a thick compact surface skin on liquid media. On agar the growth is broad, translucent ochereous yellow, concentrically marked. It comes off in pieces under the needle and is difficult to transfer.

77. *Bacillus rubidus* (Eisenberg).

Occurrence. (1) As a contamination. (2) Three times in the air of the college yard near 59th street. (3) In a trap of the carnivorous water plant *Utricularia vulgaris* in Dead Creek, Plattsburgh, N. Y.

Morphology. Small rounded bacilli $.5-.7 \times .6-1 \mu$, singly or in short chains, not motile except No. (3) which was motile when fresh but not in older cultures.

Biology. Gelatin quickly liquefied, but in No. (1) not for seventy days and then only a dry hollow was found. Milk not coagulated usually, but in a culture of No. (3) there was coagulation or boiling in fourteen days, but not subsequently. In another of No. (3) there was a partial pastry coagulum in twenty-eight days on boiling. Nitrate not reduced or very slightly. The growth on agar varies in color, but is of a translucent reddish brown shading into yellowish, orange or whitish.

Remarks. These cultures are not all alike, as will be noticed, and do not correspond entirely with Eisenberg's description, but I have preferred to regard them as varieties of one species.

78. *Bacillus delta* n. sp.

Occurrence. From a water plate.

Morphology. Short bacilli, $.5 \times .8-1 \mu$, singly and in short chains, not motile.

Biology. Gelatin slowly liquefied, beginning in twenty-one days. Milk not coagulated on boiling, though it may appear somewhat so before. Rosolic acid not changed. On agar the growth is so thin that its red color is scarcely apparent, but it is evident on milk at the surface. Grows well at $37\frac{1}{2}^{\circ}\text{C}$, of a translucent light pink. On potato shining, light red, not greatly spreading.

79. *Bacillus fulvus* (Zimmermann).

Occurrence. (1) In the air of the college hallway. (2) In the air of an apartment house on West 69th street. (3) From a water plate.

Morphology and biology. Under the above name, I associate three cultures which agree in liquefying gelatin very slowly, usually not for twenty-one days. Lactose-litmus reddened, rosolic acid not changed, no surface growth on broth. They differ as follows: (1) $.5 \times .7-1 \mu$; indol formed abundantly; on potato a narrow rather shining bright yellow growth; lactose-litmus permanently reddened. (2) $1 \times 1.2 \mu$; scarcely any indol formed; no growth on potato; lactose-litmus permanently reddened. (3) $.4 \times .5 \mu$; considerable indol is formed; on potato a coarse densely granular dry growth, brown centrally, yellow on the edges; lactose-litmus made red, but later becomes blue.

Remarks. I am unable to decide whether these are distinct species or varieties

80. **Bacillus violaceus sacchari** (Ager).*

Occurrence. In the air of the college yard.

Morphology. Short bacilli $.5 \times .7-1 \mu$, singly or in short chains, actively motile.

Biology. Gelatin quickly liquefied, milk coagulated, rosolic acid faded. Produces a green fluorescence and also a blackish color in the presence of glucose, lactose, glycerine and in some samples of broth, also on the addition of formaline.

Remarks. Differs from No. 75 in producing the violaceous black pigment. This is very well marked in old cultures in milk.

81. **Bacillus Hudsonii** n. sp.

Occurrence. (1) In the air of the college hallway. (2) A contamination from the air of the laboratory. (3) Twice in the air of the college yard. (4) A culture obtained by Prof. G. H. Hudson at Plattsburgh, N. Y.

Morphology. Small rounded bacilli, $.5-.6 \times .7-1.5 \mu$, singly or in pairs; motile.

Biology. Gelatin quickly liquefied, milk coagulated after 14 days, finally forming a distinct curd which is sometimes dissolved. Nitrate not reduced or very slightly; rosolic acid not changed. On potato the growth is thin, translucent ochereous or orange tinted. The amount of reddening of lactose-litmus varied somewhat in the different cultures. On glycerine agar an abundant growth is formed, thick, but translucent or of a mustard color, while a considerable accumulation of orange pigment collects in the condensation water in the bottom.

82. **Bacillus oxy lacticus** n. sp.?

Occurrence. (1) "Bacillus oxy lacticus" from Král's laboratory. (2.) In the air of the college yard.

Morphology. Rather large bacilli $1-1.3 \times 1.7-2.5 \mu$, singly or in long chains; not motile.

Biology. Gelatin quickly liquefied, nitrate not reduced, rosolic acid not changed; on potato the growth is shining watery translucent, somewhat marked with more opaque white. Growth on agar white with a faint ochereous yellow tint.

Remarks. I have seen no description of "Bacillus oxy lacticus."

83. **Bacillus epsilon** n. sp.

Occurrence. In the air of the college yard.

Morphology. Small rounded bacilli $.5 \times .7-1 \mu$, not motile.

Biology. Gelatin rather quickly liquefied, nitrate not reduced, rosolic acid not faded; growth on agar translucent pink, shining.

84. **Bacillus zeta** n. sp.

Occurrence. In the air of the college hallway.

Morphology and biology. As in No. 83 but the growth is shining orange color. Growth is slow and liquefaction does not begin before ten days; on the surface of milk a layer of red cream forms.

*New York Medical Journal, 1894, p. 265.

85. **Bacillus Fischeri** (Beyerinck.)

Occurrence. (1) "*Photobacterium phosphorescens*" from Krål's laboratory. (2) "*Photobacterium balticum*" from Krål's laboratory. (3.) "*Photobacterium fischeri*" from Krål's laboratory. (4) "*Photobacterium Pflügeri*" from Krål's laboratory.

Morphology. Short bacilli $.5-.6 \times .6-1.2 \mu$, usually singly.

Biology. Gelatin liquefied slowly, usually in twenty-eight days. Milk coagulated but very slowly and often not demonstrable in twenty-eight days; in No. (4) in one hundred and seventy days; nitrate slightly reduced but very slowly. Growth on solid media is yellow, but it takes place slowly and the cultures are liable to die. Rosolic acid and lacose-litmus are unchanged.

Remarks. Apparently differs from *B. argenteo phosphorescens liquefaciens* Katz only in liquefying geletin much more slowly.

86. **Bacillus proteus vulgaris** (Hauser.)

Occurrence. "*Proteus vulgaris*" from the college collection.

Morphology. Large rounded bacilli, $.8 \times 1-2 \mu$, singly and in long chains, the elements separated by spaces; spasmodically motile.

Biology. In lactose-litmus gelatin little wandering colonies may be seen in the unliquefied part of the medium; growth on agar soft, white; scarcely a well defined surface growth on broth. Gelatin, quickly liquefied, milk coagulated, nitrate not reduced.

87. **Bacillus alvei** (Cheshire & Cheyne.)

Occurrence. From the college collection.

Morphology. Slender straight bacilli $.7 \times 3.5 \mu$, in short chains, motile, forms spores.

Biology. Gelatin quickly liquefied, milk coagulated, nitrate slightly reduced. Growth on agar very thin, spreading. Rosolic acid not changed.

88. **Bacillus megaterium** (De Bary).

Occurrence. From the college collection.

Morphology. Slender bacilli, $.8 \times 2-5 \mu$, when about to produce spores the cells are more rounded and about $1-1.2 \times 4 \mu$, singly and in chains, not motile.

Biology. Milk was not coagulated in the first sample, but quickly in second one, nitrate not reduced. A wrinkled surface skin on broth.

89. **Bacillus subtilis** (Ehrenberg).

Occurrence. From the college collection.

Morphology. Large square ended bacilli, $.7 \times 2-3 \mu$, singly or in long chains; spasmodically motile, from spores which are wider than the rods.

Biology. As in No. 88, but the growth has a more crusty texture. The same contradictory effect was produced in milk as in No. 88, but this may be due to differences in the samples of milk which were the same for these two species.

90. **Bacillus anthracis** (Pollender).

Occurrence. From the college collection.

Morphology. Large square ended bacilli, $.8-1 \times 2-5 \mu$, singly or in chains, the elements often separated by spaces; not (or spasmodically?) motile; forms large spores.

Biology. Gelatin quickly liquefied, milk rapidly coagulated, the coagulum slowly dissolved, nitrate completely reduced in two days; rosolic acid not changed; lactose-litmus not reddened.

91. **Bacillus crystalloides** n. sp.

Occurrence. A contamination on some plates of *B. lactis erythrogenes*.

Morphology and biology. As in No. 90, but the growth on agar is at first rather clear, refracting, checkered-crystalline; the edges with shallow lobes.* Later it becomes thicker and opaque, coarsely granular, a little wrinkly, but on the thinner edges the crystalline markings persist.

92. **Bacillus ramosus** (Frankland).

Occurrence. (1) From Krål's laboratory. (2) "*Bacillus anthracoides*" from Krål's laboratory. (3) In the air on West 59th Street near the college. (4) With No. 91.

Morphology and biology. As in Nos. 90-91, but the growth on agar is smooth, finely granular and somewhat translucent, well defined and granular at the margin or a little feathery, scarcely at all creased.

93. **Bacillus lactis albus** (Löffler).

Occurrence. (1) Found by Dr. Kitchell in a mouse dead of anthrax. (2) In the air of the college hallway. (3) With No. 91.

Morphology and biology. As in Nos. 90-92. The cells are not motile or spasmodically so. In the media a yellow color may be produced of greater or less intensity, but the growth is not colored. The agar growth is as in No. 92, but usually wrinkled, forming elevated, sharp folds.

Remarks. Löffler has described some species of the anthrax group, and I apply his name to this form. The slower rate of liquefaction which he mentioned may be due to a difference in the composition of the media.

94. **Bacillus mycoides** (Flügge).

Occurrence. From the college collection.

Morphology and biology. As in Nos. 90-93, but the growth on agar is extraordinarily feathery and marked with concave creases.

Remarks. Nos. 90-94 may be but races of one species.

95. **Bacillus mesentericus vulgaris** (Flügge).

Occurrence. (1) From the college collection. (2) From the air in the college hallway. (3) A contamination in a culture of *B. helvolicus*.

Morphology. Large, rather square-ended bacilli, $.7-1 \times 1-3 \mu$, singly or in long chains, not or spasmodically motile, form spores.

* Dr. Cheesman has obtained this identical growth from cultures of *Bacillus anthracis*.

Biology. Gelatin quickly liquefied, milk coagulated, but usually not till after some time; nitrate partly reduced, not completely; lactose-litmus not reddened. Growth on agar translucent white with a marked tendency to spread over the surface in a very thin layer.

96. **Bacillus ochraceus** (Zimmermann).

Occurrence. From Krål's laboratory.

Morphology. Slender bacilli, $.4 \times 1-3 \mu$, singly or in chains separated by spaces; not motile.

Biology. As in No. 95, but the growth is very different, being opaque, orange yellow streaked with white, not spreading widely.

Remarks. This species is said to be motile, "slow and serpentine," but my cultures did not show it.

97. **Bacillus prodigiosus** (Ehrenberg).

Occurrence. From the college collection.

Morphology. Small rounded bacilli, $.5 \times 1 \mu$, mostly singly, actively motile.

Biology. Gelatin quickly liquefied, nitrate completely reduced; lactose-litmus reddened. The cultures on agar were viscid.

Remarks. This species is said to form gas in presence of sugar, but my cultures did not, and it seems more likely that this species and *B. rosaceus metalloides* (No. 113) have been confounded, as they are closely alike except for the gas formation.

98. **Bacillus indicus** (Koch).

Occurrence. "Bacillus indicus ruber," from the college collection.

Morphology and biology. As in No. 97 except that the growth is not viscid. (Compare Nos. 113 and 114.)

Remarks. The culture before me has become white. This form is not to be distinguished from the white variety of No. 97. I do not know whether its chromogenic form is the same or not.

99. **Bacillus violaceus laurentius** (Jordan).

Occurrence. (1) "Bacillus violaceus," from Krål's laboratory. (2) "Micrococcus violaceus," from Krål's laboratory.

Morphology. Small bacilli, $.5 \times 1 \mu$, singly or in long chains, motile.

Biology. Gelatin quickly liquefied, milk coagulated, nitrate completely reduced and rapidly; lactose-litmus made blue, rosolic acid not changed; forms indol; scarcely any growth on potato.

Remarks. This can not be *B. violaceus* Frankland, as it was not observed to form spores. It differs from *B. lividus* Plagge and Proskaner in liquefying gelatin rapidly. It differs from *B. jacinthus* Zopf, in coagulating milk, while the growth is not tough, but agrees with it in reducing nitrate quickly. It differs from *B. violaceus laurentius* Jordan in reducing nitrate quickly, in producing the violet color in ordinary broth and in not growing on potato; but I have not felt justified to consider these differences as specific.

100. **Bacillus mesentericus fuscus** (Flügge).

Occurrence. (1) From the college collection. (2) "Bacillus disciformans," from Král's laboratory. (3) A contamination in a milk culture of No. 10.

Morphology. Rather square-ended bacilli, $.5 \times .9-4 \mu$, singly or in chains, motile, form spores.

Biology. Gelatin quickly liquefied, nitrate only partially reduced, often only very slightly; lactose-litmus not reddened, rosolic acid not changed. Growth on agar soft, when moderately old pale creamy or ochreous in tint, inclined to be lobed on the edges. Forms a crusty partial skin on broth.

101. **Bacillus m. fuscus granulatus** n. sp.

Occurrence. Abundantly in a jar of "sterilized milk" with No. 1.

Morphology and biology. As in No. 100, but the growth on agar is rather coarsely granulated, and nitrate is completely reduced in twenty-eight days.

102. **Bacillus m. fuscus consistens** n. sp.

Occurrence. A contamination in a milk culture of No. 10 with No. 100.

Morphology and biology. As in No. 100, but little wandering colonies were seen in lactose-litmus gelatin in the unliquefied part of the medium as in *B. proteus vulgaris*, the bacilli in the chains were separated by well-marked spaces and the growth on agar was creased and irregular, the edges turning down, almost cutting into the surface of the medium, and was very coherent and difficult to plant. For the hanging drop, it was necessary to break up the growth with two needles.

Remarks. Nos. 101 and 102 may be varieties of No. 100.

103. **Bacillus pyocyaneus** (Gessard).

Occurrence. "B. pyocyaneus" from the college collection.

Morphology. Small rounded bacilli $.5 \times .7-1 \mu$, singly or in short chains; motile.

Biology. Gelatin quickly liquefied, nitrate completely reduced, rosolic acid rather deepened in color, lactose-litmus made blue.

104. **Bacillus lactis erythrogenes** (Hueppe).

Occurrence. (1) From the college collection. (2) "Bacillus versicolor" from the college collection.* (3) Thirteen times in the air of the college hallway and the yard.

Morphology. Very short bacilli, $.6-.9 \times .9-1 \mu$, singly in twos or short chains; not motile.

Biology. Gelatin quickly liquefied, milk apparently coagulated but without forming a curd on boiling, nitrate reduced completely, or more rarely

* Dr. Prudden's description of *B. versicolor* seems rather to apply to No. 105 and his name has precedence over Zimmermann's. Probably both forms were before him and the more quickly liquefying one got preserved as the typical culture.

only partially; lactose-litmus made blue, rosolic acid not changed. Growth on solid media soft, thick, yellow, with a pink tint in the medium.

105. **Bacillus helvolus** (Zimmermann).

Occurrence. (1) From Král's laboratory. (2) "Staphylococcus cereus flavus" from the college collection. (3) A contamination from the air of the college laboratory. (4) From the air of an apartment house on West 69th Street. (5) From air of the college hallway.

Morphology. As in No. 104, or the cells a little longer.

Biology. Gelatin slowly liquefied, sometimes not for thirty or forty days, but the character is variable; milk not coagulated; nitrate not reduced, slightly reduced, or even completely reduced, but usually not for some time. Growth on agar yellow with or without a pink tint in the medium.

Remarks. This form grades into the preceding. Zimmermann does not mention the pink pigment and it was not visible in my cultures from Europe at first, but came out some three months afterwards. My measurements are all shorter than those given in the books as the preparations were all made from agar cultures.

106. **Bacillus helvolus granulatus** n. sp.

Occurrence. Found in the process of purifying a culture of No. 105.

Morphology and biology. As in No. 105, but the growth on agar is granular lumpy, pale yellow, with scarcely any pink tint.

107. **Bacillus erythrogenes rugatus** n. sp.

Occurrence. This is the "wrinkly form" of No. 104 with which the experiments on discontinuous variations hereinbefore recounted were carried on.

Morphology and biology. It differs from No. 104 in that growth on agar is thin, skinny and covered with coarse wrinkles. (See figure.)

Remarks. This comes near *B. plicatus* Zimmermann, but liquefies gelatin much more rapidly, besides producing the pink color in media.

108. **Bacillus eta** n. sp.

Occurrence. In the air of the college hallway.

Morphology. Small rounded bacilli, $.5 \times .7-1 \mu$, mostly singly; not motile.

Biology. Gelatin liquefied slowly (in twenty-eight to one hundred days), milk not coagulated, nitrate completely reduced, but slowly; rosolic acid not changed. On solid media, yellow, viscous, with no pink tint.

109. **Bacillus fluorescens liquefaciens** (Flügge).

Occurrence. (1) From Král's laboratory. (2) "Bacillus pyocyaneus" from the college collection.

Morphology. Short rounded bacilli, $.5 \times .7-1.2 \mu$, mostly singly, actively motile.

Biology. Gelatin quickly liquefied, milk not coagulated, but the casein is dissolved; nitrate well reduced but not completely. Lactose-litmus not reddened; rosolic acid decolorized.

110. **Bacillus fuscus liquefaciens** n. sp.

Occurrence. (1) "Bacillus fuscus" from Král's laboratory. (2) A contamination from the air of the college laboratory.

Morphology. Slender bacilli. $.5-.6 \times 1-2 \mu$, singly or in short chains; not motile.

Biology. Gelatin liquefied slowly (in fourteen to fifty days), milk not coagulated, nitrate partly reduced. Lactose-litmus made blue, rosolic acid not changed.

Remarks. Differs from No. 53 only in that gelatin is ultimately liquefied. This comes near the description of *B. tremelloides* Schottelius, but the growth is never shining and the liquefaction is much slower.

111. **Bacillus theta** n. sp.

Occurrence. In the air of the college hallway.

Morphology. Rounded bacilli, $.5-.7 \times 1-1.3 \mu$, mostly in pairs; motile(?)

Biology. Gelatin slowly liquefied (twenty-one days), milk not coagulated; nitrate completely reduced in twenty-one days; lactose-litmus made blue. Growth on agar translucent ocherous, obscure. On broth, a surface skin gradually forms which may be made to sink entire. On potato, abundant shining brownish ocherous, thick and spreading.

Remarks. This seems related to No. 76, but nitrate is reduced and the growth is not just the same.

112. **Bacillus kappa** n. sp.

Occurrence. From a sick larva of *Scoliopteryx libatrix* with No. 75.

Morphology. Little rounded bacilli, singly and in pairs, $.7 \times 1 \mu$, not positively motile.

Biology. Gelatin liquefied in twenty-eight days; nitrate completely reduced. Lactose-litmus made blue. Growth white, soft, moderately opaque, not chromogenic. Forms a surface skin in the open arm of the fermentation tube.

GAS-FORMING BACILLI.

Synopsis of species.

Gelatin ultimately liquefied.

Rosolic acid faded.

Growth not viscous 113

Growth viscous 114

Rosolic acid not changed.

Lactose-litmus reddened, but ultimately becomes blue.

Agar growth opaque white.

Not motile 115

Motile 116

Agar growth translucent, white, thin, sometimes lobed, spreading.

Colonies distorted, Proteus-like 117

Colonies even, normal. *

Not or spasmodically motile 118

Actively motile 119

Lactose-litmus made blue at first	120
Gelatin not liquefied.	
Lactose-litmus permanently reddened.	
Growth fluid; cells in capsule	121
Growth not fluid.	
Nitrate partly reduced	122
Nitrate completely reduced	123
Lactose-litmus reddened, later blue.	
Rosolic acid not changed.	
Culture not viscous	124
Growth very viscous	125
Rosolic acid faded	126

113. *Bacillus rosaceus metalloides* (Dowdeswell).

Occurrence. (1) "*Bacillus miniaceus*" from Krål's laboratory. (2) "Red water from Brooklyn," from the college collection. (3) "*Bacillus magenta*," from the college collection. (4) "*Bacillus ruber* of Kiel" from Krål's laboratory.

Morphology. Small rounded bacilli, $.5-.7 \times .8-1.3 \mu$, singly, in pairs or short chains, motile, or partially so, often not motile. In old cultures, small spherical cells occur, gradually to the exclusion of the bacillar forms.

Biology. Gelatin liquefied, but not quickly; in fourteen days it is usually distinct, but may not ensue for thirty days. Milk is coagulated more or less rapidly, in all cases by twenty-one days, on boiling. Nitrate strongly reduced, usually completely. Lactose-litmus reddened. Gas is formed in the fermentation tube, and in cultures on lactose-litmus gelatin. A surface crust on broth. Rosolic acid decolorized. Growth on all media strongly chromogenic, fine crimson red, but a white form readily occurs on repeated cultivation.

114. *Bacillus Plymouthensis*.

Occurrence. "*B. rubrum*, Plymouth," from the college collection.

Morphology and biology. As above, except that the growth is viscous.

Remarks. This form is scarcely deserving of specific rank.

115. *Bacillus oxytocus perniciosus* (Flügge).

Occurrence. "*Oxytocus perniciosus*," from the college collection.

Morphology. Short, thick, rounded bacilli, $1 \times 1.2-2 \mu$, singly or in pairs; not motile.

Biology. Gelatin liquefied, but not before thirty-five days; milk coagulated; nitrate well reduced; lactose-litmus reddened.

Remarks. Described as not liquefying gelatin, but the liquefaction occurs so late that it may have been overlooked.

116. *Bacillus Kralii* n. sp.

Occurrence. "*Bacillus butyricus*" from Krål's laboratory.

Morphology. Short rounded Bacilli, $.7 \times .8 \mu$, mostly singly, motile, but not actively so.

Biology. Gelatin liquefied in about thirty days ; milk coagulated, nitrate well reduced but not completely.

Remarks. This cannot be *B. butyrus* Pragmowski which is anaërobic. It differs from *B. butyrus* of Hueppe in the size of the cells, the rate of liquefaction of gelatin and in other details. It apparently differs from *B. gasoformans* Eisenberg in liquefying gelatin much more slowly.

117. **Bacillus larvicida** n. sp.

Occurrence. In the exudate from the anus of a sick larva of *Clisiocampa fragilis*.

Morphology. Short rounded bacilli $.8 \times 1 \mu$, singly, and actively motile.

Biology. Gelatin liquefied in fourteen to twenty-one days ; milk coagulated ; nitrate completely reduced. Colonies on gelatin present curious twisted shapes like those described for the species of "Proteus."

118. **Bacillus lactis aerogenes** (Escherich).

Occurrence. From the college collection.

Morphology. Small rounded bacilli, $.6 \times 1 \mu$, singly or in pairs ; occasionally motile.

Biology. Gelatin liquefied in fifty days or not ; milk coagulated, nitrate partly reduced. One culture upon lactose-litmus gelatin produced liquefaction in fifty days and the reddening that first took place was succeeded by blue ; a second culture produced only reddening and no liquefaction.

Remarks. The second culture on lactose-litmus was not to be distinguished from *B. coli communis*. *B. lactis aerogenes* is said not to liquefy gelatin and be not motile, but these characters were not very markedly contradicted by the present culture, so I have preferred not to change the name.

119. **Bacillus vernicosus** (Zimmermann).

Occurrence. (1) From Krål's laboratory. (2) In the air of the college hallway.

Morphology. Small bacilli, $.5 \times .6-1 \mu$, singly and in short chains, actively motile.

Biology. Gelatin slowly liquefied, in from ten to thirty days, milk coagulated, nitrate not reduced or but very slightly.

Remarks. The cultures obtained by me from the air does not correspond exactly with the culture of *B. vernicosus* ; the growth has an ochreous tinge and seems otherwise slightly different. I cannot positively differentiate it, however.

120. **Bacillus pyogenes foetidus liquefaciens** n. sp.?

Occurrence. From Krål's laboratory.

Morphology. Small bacilli, $.6 \times 1 \mu$, singly or in pairs ; not motile.

Biology. Gelatin quickly liquefied, milk coagulated, nitrate not reduced. Colonies regular, uncharacteristic. Rosolic acid not decolorized.

Remarks. I have seen no description of this species.

121. **Bacillus Pruddeni** n. sp.

Occurrence. Found by Dr. Prudden in a case of Cystitis, the cells surrounded by a capsule.

Morphology. Rather large short bacilli, $.9-1 \times 1.2 \mu$, usually singly; not motile.

Biology. As in *B. coli communis*, with the exception that the growth has a soft consistency, so that an oblique culture on lactose-litmus gelatin it flows down to the base of the tube, but produces no liquefaction.

Remarks. This may be one of the many varieties of the following species, but I have not found a good description which covered the differential character which I have observed. The capsules did not appear so as to be noticed in preparations from an agar culture. Apparently this species is much like the "Pneumococcus" of Friedlander.

122. **Bacillus coli communis** (Escherich).

Occurrence. (1) From the college collection. (2) "Bacillus neapolitanus" from Krål's laboratory. (3) "Bacillus cavidica" from Krål's laboratory. (4) "Bacillus der Fretchenseuche" from Krål's laboratory. (5) "Bacillus acidi lactici" from the college collection. (6) "Proteus hominis" from the college collection.

Morphology. Short bacilli; some constricted, $.6 \times .8-1.2 \mu$, usually singly, not motile.

Biology. Milk coagulated, nitrate only partially reduced in twenty-eight days; rosolic acid not changed; growth not viscid.

123. **Bacillus Bookeri.**

Occurrence. Found by Dr. Prudden in a case of Cystitis.

Morphology. Very short bacilli, resembling cocci, $.8 \times 1 \mu$, partially motile.

Biology. Differs from the preceding in reducing nitrate completely.

Remarks. This may be the *Bacillus* f. of Booker which seems to be an unusually vigorous variety of *B. coli communis*.

124. **Bacillus acidiformans** (Sternberg).

Occurrence. From the college collection.

Morphology. Very short bacilli, resembling cocci, $.6 \times .8-1 \mu$, not motile.

Biology. Milk coagulated, nitrate partially reduced, rosolic acid not changed. Lactose-litmus is at first reddened, but in twenty-five days a blue spot appears which gradually spreads throughout the medium (ninety days).

125. **Bacillus capsulatus** (Pfeffer).

Occurrence. (1) From the college collection. (2) "Bacillus synxanthus" from Krål's laboratory.

Morphology. Bacilli varying considerably in size, $.5-1 \times .7-1.5 \mu$, singly or in short chains; not motile; no capsule seen.

Biology. Milk coagulated, nitrate completely reduced. The growth on agar is very viscid.

Remarks. Apparently *B. synxanthus* Schröter is the same as *B. capsulatus* Pfeffer. At any rate, I have been unable to differentiate these two cultures. Neither showed the capsule from agar cultures.

126. **Bacillus sordidus** n. sp. ?

Occurrence. "Micrococcus sordidus" from Král's laboratory.

Morphology. Short bacilli, $.6-1 \times 1-1.5 \mu$, singly or in pairs, rarely a short chain; not motile. In one preparation the bacilli were seen to be surrounded by a transparent capsule.

Biology. Milk coagulated, nitrate partly reduced, rosolic acid decolorized. The agar growth is very viscous and that on lactose-litmus gelatin forms a great jelly-like mass which may collect to a large extent in the bottom of the tube.

Remarks. I have seen no description of this species.

COMPOSITION OF THE MEDIA.

The following formulæ will explain the composition of the media used in the preceding investigations. All media were rendered alkaline to litmus to such a degree that they were still acid to phenol-ptalein, requiring from .1 to .2 cc. of tenth normal sodium hydrate* for every cubic centimeter of the medium to render it neutral to the latter indicator. But if the medium, as first prepared, turned out more alkaline than this, no acid was added to it. The ordinary method of titration was used.

BROTH.

Extract of meat (Liebig's), ... 5 gr.
Salt (NaCl), 5 gr.
Pepton, 10 gr.
Water (filtered), 1000 cc.
Two eggs (to clear).

GELATIN.

Broth as above prepared, with the addition of 10 per cent. gelatin.

AGAR.

Broth as above with 1 per cent. agar-agar.

GLYCERINE AGAR.

Agar with 6 per cent. glycerine.

LACTOSE-LITMUS AGAR.

Agar as above with 2 per cent. lactose and litmus enough to render distinctly purple. The litmus should be added as late as possible, as long boiling with the medium injures the color.

FERMENTATION BROTH.

Pepton, 10 gr.
Salt, 5 gr.
Glucose, 20 gr.
Water, 1000 cc.

MILK.

Commercial milk, rendered alkaline if necessary.

* 4 grams of pure sodium hydrate to 1 liter of distilled water.

NITRATE SOLUTION.

Pepton,1 gr.
Potassium nitrate (KNO_3), ..0.2 gr.
Water, 1000 cc.

ROSOLIC ACID BROTH.

Pepton,10 gr.
Salt,5 gr.
Water, 1000 cc.
Rosalic acid solution,40 cc.

(Rosolic acid solution made of rosalic acid $\frac{1}{2}$ gr., alcohol, 80 per cent., 100 cc.)

PEPTON SALT BROTH.

Pepton,1 gr.
Salt,1 gr.
Water,1000 cc.

POTATO.

Ordinary potato, sliced, boiled and rendered alkaline.

